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Biomanipulation for eutrophication control in running waters: top-down effects on benthic key stone grazers

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Übereinstimmungserklärung

Die Übereinstimmung dieses Exemplars mit dem Original der Dissertation zum Thema:

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At	Attached/motile diatom species
Apr	April
Aug	August
BL	Body length
C	Control
Ch	Chain/rosette forming diatom species
Chl *a*	Chlorophyll *a*
Con	Control stream (*Tännichtgrundbach*)
CPOM	Coarse organic matter
Dec	December
Dif	Difference phase
dw	Dry weight
Feb	February
Fi	Filamentous algal species
FPOM	Fine particulate organic matter
fw	Fresh weight
H	High
I	Intermediate
Ind	Individual(s)
Jan	January
Jul	July
Jun	June
L	Low
Mar	March
max.	Maximum, maximal
min.	Minimum, minimal
n	Number of sampling occasions
Nov	November
Oct	October
p	Significance level
P	Phosphate
PAR	Photosynthetic active radiation
RIA	Randomised intervention analysis
St	Stalk/tube forming diatom species
SD	Standard Derivation
SE	Standard Error
Sep	September
Sim	Similarity phase
SRP	Soluble reactive phosphorus
Tre	Treatment stream (*Gauernitzbach*)
TP	Total pigment (phaeopigment and chlorophyll *a*)

1 Introduction

An assessment of top-down effects on benthic key stone grazers, and, indirectly, the consequences for the periphyton community in running waters, require extensive studies of trophic interactions between dominant grazers species (e.g. mayfly larvae), their food resource periphyton and their predators (vertebrate and invertebrate predators). Trophic interactions in food webs of lakes and reservoirs have already been studied, and therefore the cascading effects of fish predation in pelagic communities are well understood. Accordingly, control of pelagic food web structures by biomanipulation is a tool for water quality management in standing waters today (Benndorf, 1990; Jeppesen et al., 1997; Hansson et al., 1998). The knowledge of principles and processes involved in structuring benthic grazer and periphyton communities is a necessary precondition for implementation of top-down acting key mechanisms in stream food webs. In addition, the grazing performance itself and, consequently, the potential role of benthic grazers in controlling excessive and nuisance periphyton growth in running waters are also crucial requirements to understand ecosystem relationships. However, the prospective transfer of the biomanipulation concept to running water ecosystems needs further research on predator-grazer and grazer-periphyton interactions including related indirect effects. The overall objective of this approach, as a part of eutrophication management, is the mitigation of eutrophication consequences in streams and rivers.

1.1 Background and motivation

1.1.1 Eutrophication and biomanipulation of streams

Eutrophication of running waters is one of the most prevalent environmental problems throughout the world. Eutrophication is responsible for water quality degradation, structural and functional ecosystem alterations and, finally, for the compromise of ecosystem services (Dodds et al., 2008; Istvanovics and Honti, 2012). Intensification of agriculture is the major source of external nutrient loads (primarily nitrogen and phosphorus) to aquatic ecosystems today causing freshwater eutrophication (Carpenter et al., 1998; Smith, 2003). Also in small deciduous, forested streams, like the investigated ecosystems in this study, intense eutrophication effects can be observed particularly in spring because of three effects:

- missing foliation of riparian canopy,
- increasing light intensity due to longer length of daytime and
- high nutrient supply from agriculturally used catchments during this period (Dodds, 2007).

However, anthropogenic nutrient enrichment of streams and rivers can generate predictable increases in biomass and abundance of primary producers such as benthic algae and macrophytes (Harper, 1992; Biggs, 2000; Wetzel, 2001). Large algal biomasses constitute a nuisance to water quality, recreational use and water supply (Freeman, 1986). Additionally, large portions of periphyton, particularly filamentous algae, can detach or be removed by hydrodynamic forces. The consequential clogging of sediment beds leads to decreased permeability of the stream bed and to an increased oxygen demand in the bottom substrata, thus degrading benthic habitats of streams (Welch et al., 1989; Ibisch and Borchardt, 2003). Such degraded streams are colonised with a less diverse, pollution-tolerant benthic community (Armitage et al., 1983). It is suggested that algal material can mechanically clog the porous channels of the hyporheic zone and that algal exudates can lead to an increase of heterotrophic biofilms and thereby enhance the clogging process (Battin and Sengschmitt, 1999; Ibisch and Borchardt, 2003). Intact riverbed sediments, however, are the key to ensure important ecological functions in running water ecosystems, for example:

- self-purification,
- refuge against adverse conditions,
- spawning and hatchery habitat which take place exclusively in the hyporheic zone (Brunke and Gonser, 1997; Ibisch et al., 2009).

The reduction of external nutrient input through nitrogen (N) and phosphorus (P) is the key factor for eutrophication control in lakes and reservoirs (Smith, 2003). However, eutrophication management in running water ecosystems faces additional challenges due to the difficulty in understanding important ecosystem-internal control mechanisms, the larger catchment areas and the dynamics of running waters itself (e.g. dynamically changing physical, chemical, biological gradients) as 'open' ecosystems (Istvanovics and Honti, 2012). Historically, the control of eutrophication in running waters was primarily focused on the reduction of point sources of nutrient pollution such as waste water treatment plants (Chambers et al., 2008). However, diffuse pollution, particularly agricultural run-off, is difficult to control,

although it is recognised that this kind of pollution constitutes the major nonpoint source of N and P to many inland and coastal waters (Dodds, 2006). Therefore intensified efforts are needed to improve the environmental condition of running waters, for example:

- sustainable agriculture (e.g. correct management of fertilizers and manure, riparian buffer stripes, cropland terracing),
- assessment of ecological conditions of receiving waters (e.g. monitoring chemical and physical factors and effects on aquatic biota) and
- adoption of environmental quality targets (e.g. guidelines, standards, benchmarks) (Chambers et al., 2008).

Besides the control of external nutrient inputs from catchment areas, the control of internal ecological processes (i.e. ecotechnology) is known as an additional measure which can be used to reduce symptoms of eutrophication and to improve the water quality (Benndorf, 1990). A combination of ecosystem-external measures, such as reduction of nutrient import, and ecosystem–internal measures, such as food web manipulation, seems to be the most promising approach for an effective and stable reduction of freshwater eutrophication (Benndorf and Kamjunke, 1999).

Modifying the food web structure by biomanipulation is a generally accepted ecotechnological tool in water-quality management and has been studied intensively in lake ecosystems and reservoirs (e.g. Benndorf, 1990; Jeppesen et al., 1997; Drenner and Hambright, 1999). The fundamental mechanisms and processes involved in this technique are well understood within pelagic food-webs (e.g. Benndorf et al., 1988; Brett and Goldman, 1996; Benndorf et al., 2002). The approach of biomanipulation is based on the assumption that an optimal piscivorous fish stock (highest trophic level) has indirect positive impact on water quality via cascading effects. Piscivorous fish reduce the biomass of zooplanktivorous fish, resulting in an increase of abundance and size of herbivorous zooplankton. This leads to enhanced grazing pressure on phytoplankton at the lowest trophic level and, finally, causes a reduction of phytoplankton biomass (Carpenter et al., 1996; Jeppesen et al., 1999).

In stream ecosystems, however, there is a much lower level of knowledge about trophic interactions and nutrient cycling. Therefore it is not clear whether the idea of biomanipulation can be transferred to running waters and whether this biological restoration method has potential as stream management tool to improve water

quality (ecosystem-internal mechanism). For an effective biomanipulation, similar to the ones in lakes and reservoirs, it seems necessary to boost herbivorous key stone species (e.g. mayfly larvae) to enhance grazing pressure on the periphyton community by top-down acting ecosystem-internal mechanisms (Figure 1.1).

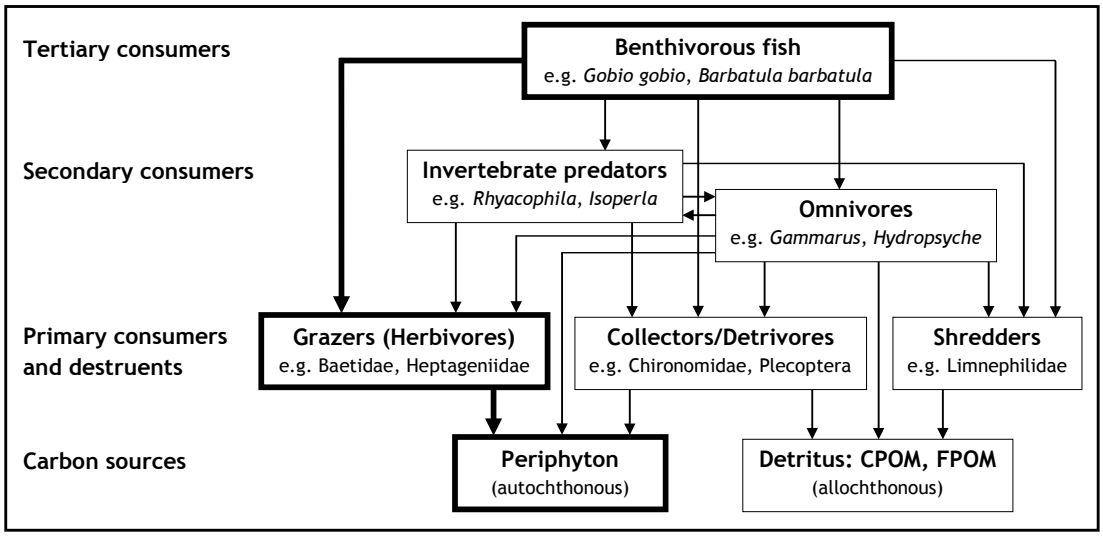

Figure 1.1 Simplified structure of a stream food web. Arrows indicate consumption. Highlighted (bold frames and arrows): top-down acting ecosystem-internal mechanisms as target for the biomanipulation approach.

The food web structure of small streams can be modified as was shown by Winkelmann et al. (2011) and Worischka et al. (2014). An experimentally increased benthivorous fish stock (gudgeon: *Gobio gobio* and stone loach: *Barbatula barbatula* as top predators, tertiary consumers) caused biomass reduction of some primary consumers (e.g. mayflies) and structural change in the upper levels of the benthic food web. Thus, a first step towards biomanipulation transfer is done because it seems possible to change food web structures in small streams. A subsequent step is necessary to clarify whether biomass and structure of the periphyton community can be top-down controlled by benthic grazing.

1.1.2 Effects of fish predation on benthic invertebrates in streams

Predation is recognised to be one of the major forces influencing population dynamics and community structure in aquatic ecosystems (Sih et al., 1985; Menge and Farrell, 1989). In stream ecosystems predatory fish are often the most important

predators. Their impact on benthic communities varies among the studies: several experiments have shown strong effects of fish predation (e.g. Gilliam et al., 1989; Power, 1990a; Bechara and Moreau, 1992), whereas others have shown weak or no effects (e.g. Allan, 1982; Culp, 1986; Reice, 1991). Many factors can be responsible for this variability such as differences in spatial scale among the studies (Englund and Cooper, 2003; Bergström and Englund, 2004), temperature (Kishi et al., 2005), habitat and substrate complexity (e.g. Lima, 1998; Townsend, 2003; Winkelmann et al., 2008) and biotic factors like interactions between multiple predators (Rosenfeld, 2000b) or between predator and prey (e.g. Hill and Harvey, 1990; Sih and Wooster, 1994; Rosenfeld, 2000a).

Predators can have lethal effects on prey communities which may result in control of prey populations or alterations of species diversity and may cause even extinctions of prey species (Kerfoot and Sih, 1987). Sublethal predation effects can have even stronger impact than lethal effects on prey population dynamics by alterations in prey behaviour, morphology or life history traits (Allan, 1981; Gerking, 1994). It can be assumed that both lethal and sublethal (mainly due to behavioural changes) predation effects reduce the grazing rate of benthic invertebrates. Prey organisms use a variety of behaviour patterns to reduce encounter rates with their predators. Typical avoidance behaviour of benthic invertebrates during fish presence includes:

- shifts in habitat use,
- changes in activity level,
- increased drift activity or
- alterations in foraging behaviour (e.g. Sih, 1980; Gilliam and Fraser, 1987; Peckarsky et al., 1993; McIntosh and Townsend, 1994; Tikkanen et al., 1994; Huhta et al., 2000; Winkelmann et al., 2008).

Despite the benefit of enhanced survivorship, these predator avoidance mechanisms are also associated with costs, such as reduced feeding rates in low quality habitats (Sih, 1987; Lima and Dill, 1990; Lima, 1998) which can result in delayed maturity or smaller size of adults at reproduction (Kohler and McPeek, 1989; Peckarsky et al., 1993; Scrimgeour and Culp, 1994). Consequently, it is believed that prey species are able to balance these potentially conflicting demands of maximising foraging success and avoiding predators (Dill, 1987; Sih, 1987; Lima and Dill, 1990; Hellmann et al., 2011).

Fish predation not only affects benthic invertebrates. Predator-induced effects can also cascade through the food web resulting in dramatic changes in abundance and biomass of lower trophic levels (Power, 1990a; McIntosh and Townsend, 1996). These indirect predation effects commonly occur in systems which are controlled by fish predation and can be caused by the consumption of benthic invertebrates and fish influencing the behaviour of their prey (Townsend, 2003; Peckarsky et al., 2008). Several studies have examined autotrophic-based food webs in running waters where fish predators negatively affect grazers, which in turn has positive effects on the primary producers. In this context most studies focused on the algal biomass response such as chlorophyll-*a* (e.g. Bechara and Moreau, 1992; Rosenfeld, 2000b; Meissner and Muotka, 2006) or ash-free dry mass (e.g. Bechara and Moreau, 1992; McIntosh and Townsend, 1996). In some cases, however, physiognomy and composition of benthic algal assemblages were considered (Hill and Harvey, 1990; Power, 1990b; Alvarez and Peckarsky, 2014). The taxonomic composition of algal assemblages differed significantly between fish and fishless streams as the proportion of total benthic algae unpalatable to grazers (non-diatoms) tended to be higher in fishless streams. Only a few species of diatoms and cyanobacteria were observed in the streams without fish whereas the algal group of chrysophytes only occurred in fish streams (Alvarez and Peckarsky, 2014).

Different types of predatory stream fish have diverse impacts on benthic prey (Dahl, 1998b). Generally, in stream ecosystems there are two types of predatory fish, drift-feeding and benthic-feeding (benthivorous) fish. While benthic-feeding fish forage mainly at or near the bottom, drift-feeding fish usually take their prey from the water column and at the water surface. Consequently, the diet of drift feeders such as salmonids may include large amounts of terrestrial invertebrate inputs which can provide up to 50 % of their annual diet and energy budget (Allan, 1981; Bridcut and Giller, 1995; Kawaguchi and Nakano, 2001; Nakano and Murakami, 2001). Changes in availability of terrestrial prey therefore can cause alteration of predation pressure by drift-feeding fish in a stream food web which potentially can cascade to the primary producers (Nakano et al., 1999; Baxter et al., 2005). It is therefore assumed that benthic-feeding fish are more effective at controlling benthic prey than drift-feeding fish because drift feeders only consume exposed food items they can detect visually, also they do not feed on interstitial prey (Power, 1992; Gerking, 1994; Dahl and Greenberg, 1996; Dahl, 1998b). Nevertheless, experiments

which exclusively used benthic-feeding fish to examine the top-down effects on benthic invertebrate communities, particularly on mayflies, are rare.

1.1.3 Effects of benthic grazers on stream periphyton

Periphyton (other terms: biofilm, *Aufwuchs*) constitutes a complex community including algae, heterotrophic organisms (protozoa, fungi and bacteria) and associated dead organic matter which adhere to submerged surfaces or move on it (Azim et al., 2005). As part of the periphyton matrix benthic algae are the dominant autochthonous primary producers in most stream ecosystems, and represent the basis of benthic stream food webs (Lamberti, 1996). Benthic algae also fulfill important functions such as removal of nutrients from the water column and provision of habitats for other benthic individuals (Stevenson, 1996). On the other hand, benthic algae can drastically reduce ecosystem quality when blooming in nutrient enriched streams, e.g. in intensively used agricultural catchments (e.g. Trotter and Hendricks, 1979; Wharfe et al., 1984; Biggs, 1985).

Periphyton growth and loss in stream ecosystems is controlled by complex interactions of abiotic and biotic factors (Feminella et al., 1989; Steinman, 1992; Rosemond, 1993), such as hydrological forces, grazing and resource availability. These proximate factors are able to control the periphyton growth and loss directly and can be classified into factors regulating periphyton accrual and counteracting factors regulating periphyton loss (Figure 1.2). The main factor for algal accrual is the availability of resources (bottom-up force), particularly light and nutrients. The main factors for algal loss are floods and grazing by invertebrates and fish (top-down force, Figure 1.2) (Biggs, 1996).

In the streams studied here benthic invertebrate community constitutes the major fraction of grazers because there were no grazing fish species. In the upper sections of Central European mountain streams there are few fish species only feeding on benthic algae. However, the Eurasian minnow (*Phoxinus phoxinus*), the European chub (*Squalius cephalus*) and the Common nase (*Chondrostoma nasus*) are known to feed on benthic algae. Some studies even showed strong grazing effects of the minnow species *Campostoma anomalum* which is not found in European streams (Power and Matthews, 1983; Power et al., 1988; Gelwick and Matthews, 1992).

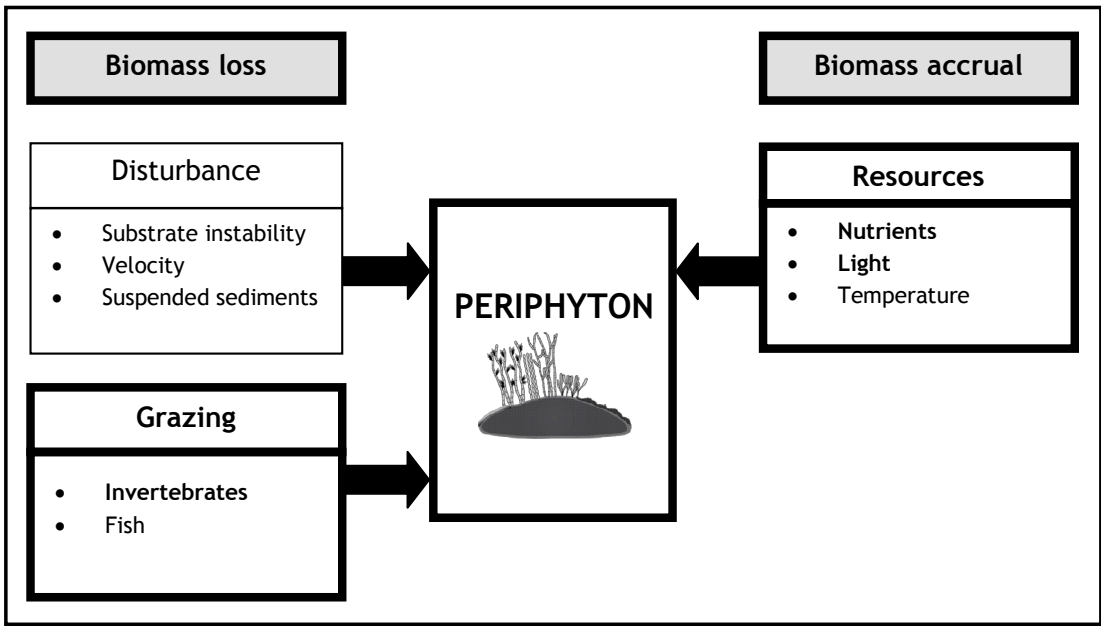

Figure 1.2 Factors controlling periphyton biomass in stream ecosystems (adapted from Biggs, 1996). Highlighted (bold frames and writing): regulatory mechanisms for periphyton loss (invertebrate grazing) and for periphyton accrual (resources light and nutrients) as subject of this thesis. Picture credits: Renate Braden (Federal Institute of Hydrology, FIH).

Benthic grazing can alter structural and functional characteristics of periphyton (Figure 1.3), for example, including:

- biomass and abundance,
- taxonomic composition,
- physiognomy and
- primary production (e.g. Lamberti and Resh, 1983; Feminella and Hawkins, 1995; Steinman, 1996; Opsahl et al., 2003; Liess and Hillebrand, 2004; Wellnitz and Poff, 2006).

In addition, benthic grazers affect the periphyton not only directly by consumption and physical disruption but also by indirect pathways, especially nutrient regeneration (Allan and Castillo, 2007a) (Figure 1.3).

The structural response of periphyton to benthic grazing has been demonstrated with many different grazers types such as snails, caddisflies, chironomids, mayflies and fish (Steinman, 1996). The outcome of grazer-periphyton interaction

varies depending on grazer species and benthic algae involved. Different grazing effects can occur due to differences of invertebrate grazers in:

- mouthpart morphology,
- mobility,
- head orientation,
- grazer density and
- behavioural adaptations (Gregory, 1983; Lamberti and Moore, 1984; Hill and Knight, 1987; Sommer, 2000; Flecker and Taylor, 2004; e.g. Álvarez and Peckarsky, 2005).

Benthic invertebrates show morpho-behavioural adaptations to periphyton-grazing and maintaining their position on exposed surfaces in fast running waters (Cummins 1979). In addition, benthic invertebrate grazers possess different types of highly specialised mouthpart morphologies and this determines the zone of the periphyton mat where they can feed most effectively.

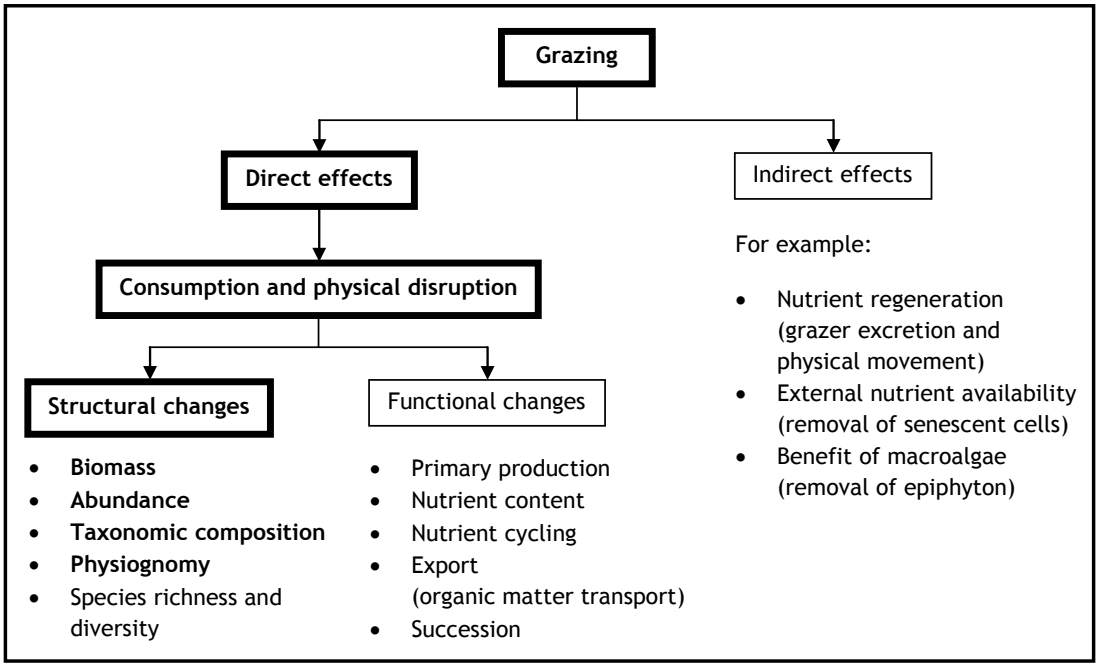

Figure 1.3 Grazer-periphyton interaction: multiple effects of benthic grazers on periphyton community in freshwater stream ecosystems. Highlighted (bold frames and writing): direct pathway of the grazing process causing structural changes of benthic algae as subject of this thesis.

Despite their specialisation grazing invertebrates have to scrape, brush off or shave stones, often for several hours per day in order to get their sufficient quantity of algal food (Arens, 1989). Different types of scraping apparatus and tools are distinguishable for algae grazers, and evidence suggests that each scraping tool is adapted to a specific kind of algal pasture (Arens, 1989). Most of the grazing mayfly larvae (Ephemeroptera) have gathering collector-feeding structures (Merritt and Cummins, 1996), and it is assumed that this grazer type harvests outer layers, or loosely attached algae of the periphyton mat (Hill and Knight, 1987; Hill and Knight, 1988). According to their scraping tools, some heptageniid mayfly larvae (e.g. *Rhithrogena semicolorata*, *Electrogena ujhelyii* and *Ecdyonurus* sp.) have evolved scraping brushes also called brushing mouthparts (Arens, 1989). The assumption is that these grazer species can feed effectively on the upper layer of algal pasture due to the fine structures of these brushes, and therefore have little impact on diatom densities (Arens, 1989; Karouna and Fuller, 1992; Holomuzki et al., 2006). In contrast, the feeding apparatus of the baetid mayfly larvae (e.g. *Baetis rhodani*) shows cutting-edged scraping tools such as long scraping gouges (Arens, 1989). It is suggested that these bladelike mandibles are suitable to reduce diatom densities particularly by scraping on hardened structures of algal pastures. These gouges are equipped with a vaulted blade or shovels which can press heavily against the substratum (Arens, 1989; Karouna and Fuller, 1992). These structures end distally in a sharp cutting edge (Arens, 1989) which can be used probably to harvest filamentous growth forms. The results of this study show that different grazing effects can occur even within one grazing type (here: grazing mayflies, Ephemeroptera) depending on their morphological traits.

The combination of morphology of grazers feeding apparatus and algal growth forms can thus be assumed to determine intensity of algal removal and susceptibility of single algal layers to grazing (Figure 1.4). A reduction of algal overstory was indicated in 37 out of 43 grazing studies whereas algal understory increased in 36 out of 41 studies (Steinman, 1996). These grazing effects could be observed also with mayfly larvae (e.g. Colletti et al., 1987; Hill and Knight, 1987; Hill and Knight, 1988; Wellnitz and Ward, 2000). Both responses can be interpreted as results of the algal species position within the periphyton assemblage. The decline of overstory growth forms is a direct consequence of their susceptibility to dislodgement due to their higher mechanical vulnerability (Hill and Knight, 1987; Hill and Knight, 1988;

Lamberti et al., 1989; Hillebrand et al., 2000). The increase of understory growth forms is the result of both direct and indirect grazing effects. Understory growth forms are not as strongly reduced because of their lower vulnerability compared to algal overstory and are indirectly facilitated by removal of overstory growth forms and consequently increase in resource availability (Feminella and Resh, 1991; Mulholland et al., 1991). Generally, reduction of algal overstory means reduction of those growth forms responsible for clogging of stream bed sediments (e.g. filamentous, chain and rosette forming algae; Figure 1.4) due to their fast detachment or easy removal by hydrodynamic forces. Therefore, change in periphyton physiognomy towards an adherent algal understory via benthic grazing could be helpful to prevent or reduce eutrophication consequences such as colmation of the hyporheic zone.

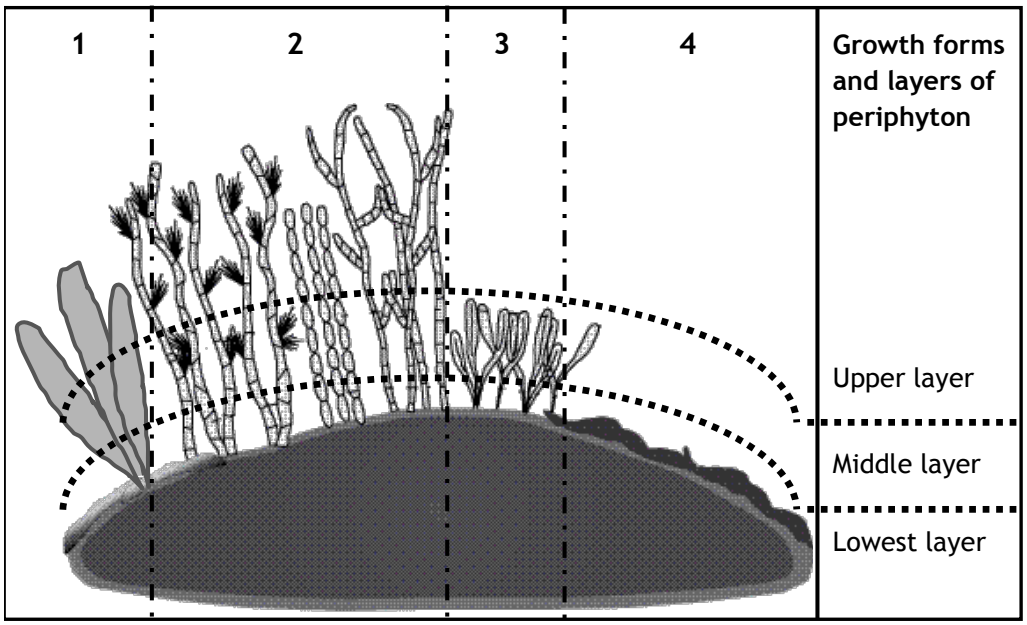

Figure 1.4 Schematic representation of main algal growth forms based on their vertical position in the periphyton community. Group 1 includes large rosette forming species and group 2 includes filamentous and chain forming algal species. Species of both groups are located in the upper layer and represent the algal overstory. Group 3 includes stalk/tube forming species (middle layer) and group 4 includes attached/motile species (lowest layer). These groups represent the algal understory (inspired by Steinman, 1996 and Katano et al., 2002). Picture credits: Renate Braden (Federal Institute of Hydrology, FIH).

Still grazers not always do reduce the algal overstory. Some studies with the filamentous algae *Cladophora* sp. (Chlorophyta) reported that grazing may increase the

biomass of these overstory algal species due to removal of epiphytes from algal filaments and due to nutrient release by the grazers (Dudley, 1992; Sarnelle et al., 1993). In addition, it is assumed that filamentous growth forms are difficult to consume or to digest for some grazers because of their size and their texture (Gregory, 1983; Lamberti and Resh, 1983; Dudley and D'Antonio, 1991). However, the mismatch between mouthpart morphology and algal physiognomy is the main reason preventing a successful alteration of periphyton physiognomy by benthic grazers.

Grazing by benthic invertebrates usually results in reduction of algal biomass (Steinman, 1996; Hillebrand, 2002; Hillebrand, 2008a). However, Hillebrand et al. (2002) pointed out that other periphyton components like bacteria, ciliate and meiofauna increased their biomass in the presence of benthic grazers indicating that grazers are not generalist consumers. Benthic grazing also affects spatial variability of periphyton biomass distribution because grazed periphyton shows a higher spatial heterogeneity than ungrazed periphyton (Álvarez and Peckarsky, 2005; Hillebrand, 2008a). The highest heterogeneity of periphyton biomass occurred in that treatment with the most mobile grazer (here: Ephemeroptera: *Baetis bicaudatis*) in a microcosm experiment (Álvarez and Peckarsky, 2005). Reasons for presence of grazers not always leading to a decline in algal biomass, for example are:

- mismatched feeding morphology of the grazers involved,
- insufficient grazer density,
- insufficient consumption rate and
- constrained biomass accrual irrespective of grazers presence or absence due to strong nutrient limitation (Steinman, 1996).

Even an increase in periphyton biomass can be a response to grazing. This occurs due to nutrient regeneration via grazer excretion, prevention of litter accumulation by physical movement of grazers and due to removal of dead or senescent cells during the grazing process leaving a greater proportion of viable cells within the periphyton matrix (Figure 1.3) (Lamberti and Resh, 1983; Lamberti et al., 1989; Swamikannu and Hoagland, 1989).

Similar to physiognomy, taxonomic composition of periphyton communities can also be affected by benthic grazing but generalisations are extremely difficult because of the enormous diversity of benthic algae in running water habitats (Steinman, 1996). A study by Rosemond et al. (2000) observed alterations in algal

species composition under grazed conditions regardless of the resource levels. This change was caused by a trade-off between grazer-resistant and not resistant but fast growing algal species. Under grazed conditions grazer resistant taxa (e.g. cyanophytes) seem to dominate the periphyton community (Steinman et al., 1987a; DeNicola et al., 1990). In this context, the tendency towards a decline of susceptible algae and an increase of resistant taxa seems to be logical (DeNicola et al., 1990; Wellnitz and Ward, 1998; Hillebrand et al., 2000). Finally, Steinman (1996) concluded that benthic grazing is able to simplify the taxonomic composition of a periphyton community.

1.2 Goals and questions

The primary objective of this study was to analyse the interaction between benthic grazers and periphyton using differently top-down controlled stream food webs and to assess the potential of the ecosystem service 'benthic grazing' as an ecotechnological tool for eutrophication control in small streams. The general hypotheses of the present thesis were:

Hypothesis 1: Periphyton biomass can be top-down controlled by benthivorous fish via invertebrate grazers**.**

Hypothesis 2: Benthic grazers will structurally alter periphyton physiognomy by removing algal overstory and establishing an algal understory canopy.

The thesis starts with long-term measurement of grazer and periphyton biomasses in the presence/absence of benthivorous fish (stone loach: *Barbatula barbatula* and gudgeon: *Gobio gobio*) as top predators in the stream food webs studied (**Chapter 2**). The dependencies of primary production (periphyton biomass accrual) and grazing rate (periphyton biomass loss) from variable environmental factors was also subject of this section. Only the observation of these rates allows estimation of optimal grazer densities, derivation of causal relationships between grazer and periphyton biomass and finally, assessment of potential effectiveness of this biomanipulation approach in running waters. Therefore a simple dynamic model was constructed to quantify bottom-up and top-down effects on periphyton by evaluating the rates of periphyton accrual and loss. The hypotheses of this investigation were:

Hypothesis 1a: The presence of benthivorous fish will reduce grazer biomass and thus increase the periphyton biomass (trophic cascade).

Hypothesis 1b: The strength of grazer-periphyton interaction will vary seasonally.

The next part of the thesis assessed the strength of trophic cascades in streams in order to quantify cascading effects of benthivorous fish (stone loach and gudgeon) via benthic invertebrate grazers (Ephemeroptera: *Baetis rhodani, Rhithrogena semicolorata, Electrogena ujhelyii, Ecdyonurus* sp.) on periphyton community composition (**Chapter 3**). The hypotheses of this section were:

Hypothesis 2a: Fish-induced changes in grazer biomass can alter the periphyton community composition due to possible biomass changes of grazer species which prefer certain algal food items.

Hypothesis 2b: The presence of benthivorous fish will change the feeding selectivity of benthic grazers.

The investigation of diel feeding periodicity and consumption rates of grazing mayfly larvae *Baetis rhodani* (Ephemeroptera: Baetidae), in response to different densities of nocturnal feeding benthivorous fish (stone loach and gudgeon), was subject of the last part of the thesis (**Chapter 4**). The goal was to identify behavioural responses of larvae to different levels of predation threat and to evaluate consequences of predator avoidance for the performance of the ecosystem service 'benthic grazing'. This part of the thesis also included the assessment of the transferability of these predator effects on natural stream ecosystems. For that purpose experimental results were compared with field observations which were obtained over a three-year period in two small mountain streams containing different densities of benthivorous fish. The hypotheses of this section were:

Hypothesis 2c: The grazer species *B. rhodani* will change its temporal feeding periodicity in order to avoid nocturnal feeding fish species.

Hypothesis 2d: Predator avoidance behaviour of this grazer species will result in reduced grazing rates.

1.3 Strategies for studying grazer-periphyton interactions in streams

The interaction between grazers and periphyton has been examined using a variety of experimental approaches, including experiments in laboratory streams, field mesocosms, and large scale field experiments. The majority of studies on grazer-periphyton interaction uses small temporal and/or spatial scales in artificial stream systems (e.g. DeNicola et al., 1990; McCormick, 1994; Wellnitz and Ward, 2000; Villanueva and Modenutti, 2004b) because of clear advantages of laboratory and mesocosm experiments, such as control of potentially important factors and the possibility to manipulate diversity and density of grazers in addition to nutrients or light. Further, sufficient replications for statistical reasons are possible in these experimental setups. Besides the obvious benefits of small-scale experiments, field experiments also are an extremely important tool to assess the transferability of obtained laboratory results to a larger scale and to study complex interactions in natural conditions.

Comparison of 89 experimental studies on grazing in streams showed that grazing effect on periphyton was less pronounced in laboratory than in field experiments (Feminella and Hawkins, 1995). One study concluded that the relationships between invertebrate grazers and periphyton can be better observed on larger than on smaller scales (Doi and Katano, 2008). Thus, it might be argued that due to the experimentally necessary reductions in time, space or complexity and the exclusion of environmental variability, laboratory experiments cannot fully determine the role of benthic grazing within streams at the ecosystem scale (Carpenter et al., 1995; Schindler, 1998; Petersen and Hastings, 2001). Field experiments require a lot of effort and long time compared to laboratory stream experiments: at least one to three years are necessary to observe changes in biomass or community composition of periphyton or benthic invertebrates. Hence, most large-scale field studies represent observations or system comparisons (Huryn, 1998; McIntosh et al., 2005; Greig and McIntosh, 2006). Although experiments focusing on the ecosystem level offer less experimental control, they explicitly include the effect of temporal and spatial variability which are important characteristics of natural conditions. Consequently, these large-scale and long-term experiments seem to allow an easy transfer of experimental results to natural ecosystems. Because this was the general aim of this

study, we decided to perform a large-scale experiment in spite of the obvious risks and shortcomings.

The large-scale field experiment used here roughly followed the "Before-After-Control-Impact" design (BACI, Stewart-Oaten et al., 1986; Carpenter et al., 1989). It was applied due to lack of replication on the stream level. The study of replicate systems is extremely important to include natural variability, but replication on the stream level in a manipulative ecosystem experiment would have caused an immense amount of work hard to manage. The study of only one or two systems is practicable in most cases (e.g. Elser et al., 1998; Raikow and Hamilton, 2001; Ferreira et al., 2006). Whole-ecosystem experiments following the paired BACI approach need at least two water bodies (here: experimental and reference/control streams) and two phases (before and after manipulation, here: fish vs. fishless) as an alternative to replication. Such experiments need an even longer experimental duration due to the study of "before" and "after" experimental manipulation period each for an equally long time span (one to three years) and, not least, finding appropriate reference sites is the crucial point of this approach (Reynoldson et al., 1997; Schmidt et al., 2009a).

Within the applied pairwise ecosystem experiment two species of benthivorous fish were used as top predators (stone loach: *B. barbatula* and gudgeon: *G. gobio*) in order to analyse the trophic interactions between fish, benthic grazers and periphyton community. One stream was used as reference/control stream containing high fish densities throughout the experiment. The other stream served as treatment stream which was experimentally manipulated with different fish densities between the subsequent years. The pair of streams was studied before and after the manipulation to quantify changes in between-stream differences. The fish stock was manipulated in both phases and in both streams to ensure the desired fish densities. Therefore there has been no reference phase in the context of natural, undisturbed conditions as in the classical BACI design. Nevertheless, the chosen statistical design was assumed to be robust because the general BACI design does not necessarily need a natural reference and does not include any directional aspects. The general design, however, supposes that the system can return to its original state at any time if the fish biomass is at the same level in both systems (resilience).

2 Top-down and bottom-up effects on periphyton

Assessment of grazer and periphyton biomass during fish presence/absence and evaluation of primary production and grazing rates via modeling

2.1 Abstract

We conducted a paired large-scale predation experiment over 32 months in two streams being seasonally shaded by deciduous riparian trees, using the benthivorous fish species stone loach (*Barbatula barbatula*) and gudgeon (*Gobio gobio*) as top predators. The biomass dependence of benthic grazers and periphyton on fish presence/absence was measured and the periphyton production was compared with the consumption rates using a model-based approach.

A three-level trophic cascade from benthivorous fish via benthic grazers to periphyton was evident from the field experiment. Integrated over the whole study period, fish reduced the biomass of the benthic grazers and indirectly increased the periphyton biomass.

Scenario analyses, using a simple dynamic model, indicated top-down control on periphyton to be strongest during autumn, when periphyton growth was light limited, and weaker in the spring, when periphyton growth was not light-limited. The seasonal light supply variation was caused by shading due to deciduous riparian trees during the vegetation period.

This asymmetry in temporal processes weakened the top-down control in a natural benthic community. Even though grazer biomass is naturally reduced in summer, due to the emergence of the most abundant species (mayflies, Ephemeroptera), a grazer biomass high enough to reduce the spring periphyton peak could not be sustained by the low summer periphyton growth.

We suppose that the temporal decoupling of grazer biomass from periphyton biomass might be caused by the very short generation time of the primary producers (days) compared to the long generation time of the primary consumers (mostly one year).

2.2 Introduction

The relative roles of top-down and bottom-up forces have long been discussed in community ecology. One broadly accepted view is the existence of top-down forces on a bottom-up template (Hunter and Price, 1992). Both forces can generate trophic cascades whereby changes to one trophic level cause adverse chain-like effects in the subsequent levels (Power, 1992). Top-down trophic cascades, from fish over benthic grazers to periphyton, have been observed in stream ecosystems (McIntosh and Townsend, 1996; Buria et al., 2010). These cascading effects seem to be possible because the trophic interaction between grazers and periphyton is particularly strong (Borer et al., 2005; Hillebrand, 2009). While benthic grazers in streams are generally assumed to reduce algal biomass, the magnitude of top-down control appears to be influenced by resource supply (Holomuzki et al., 2010). However, this assessment is mainly based on the results of small-scale experiments in laboratories or field mesocosms (Rosemond et al., 1993; Hillebrand, 2009; Holomuzki et al., 2010) while large-scale manipulations are seldom done. One major advantage of large-scale experiments is the explicit inclusion of the effect of environmental context in addition to spatial and temporal variability.

Some large-scale nutrient enrichment experiments concerned with the trophic interactions in streams have focussed on the bottom-up control of periphyton and grazers. These generally showed a positive effect of nutrient concentration on periphyton (Elwood et al., 1981) or on grazers, via periphyton (Hershey et al., 1988; Hinterleitner-Anderson et al., 1992; Hershey et al., 1993). Other studies explicitly addressed the top-down control of periphyton biomass (Yasuno et al., 1982; Kohler and Wiley, 1997; Greathouse et al., 2006; Katano et al., 2007). However, only Peterson et al. (1993) have documented top-down as well as bottom-up effects using an enrichment experiment. They observed at first the positive response of periphyton to nutrients and subsequently an increase of grazers, which limited the periphyton accrual. Studies on predation effects in streams using at least reach-scale approaches and covering multiple trophic levels are rare (but see: Huryn, 1998; Meissner and Muotka, 2006; Buria et al., 2010) and seem not to address a possible parallel bottom-up control mechanism.

Apart from factors such as predator density, predator type, nutrient supply or periphyton productivity (Biggs et al., 2000; Carpenter et al., 2001; Kurle and Cardinale, 2011), seasonal variation of environmental factors has also the potential

to affect trophic interactions in stream ecosystems. This is indicated by the fact that the direction and strength of predator-prey interactions showed seasonal pattern in a model (Sandvik et al., 2002). Predation experiments showed the influence of seasonally changing water temperature and predator responses on predator-prey interactions (Pennuto, 2003; Kishi et al., 2005). Despite an analysis of the influence of seasonal variation in grazer-periphyton interactions being still missing, the impact of several environmental factors, such as substratum heterogeneity, temperature, resource supply or hydrodynamic conditions, has been reported (Pringle and Hamazaki, 1997; Kishi et al., 2005; Peters et al., 2007; Hillebrand, 2009).

A premise of our study is that the strength of top-down effects in the benthic community of a stream can be described by the ratio of consumption and production rates. We focussed on the ratio of the periphyton biomass accrual rate ('primary production') to the rate of periphyton removal by grazers ('grazing') for the analysis of a three-level trophic cascade with indirect fish effects on periphyton because the invertebrate herbivores seem to determine the strength of a trophic cascade (Borer et al., 2005; Shurin and Seabloom, 2005). We expected predation to affect grazing directly by a reduction in grazer biomass and possibly by behavioural effects (Peckarsky, 1996). Therefore our hypothesis was that the periphyton biomass in streams can be top-down controlled by benthivorous fish via invertebrate grazers (**Hypothesis 1a**). We further assumed that the strength of grazer-periphyton interaction will vary seasonally (**Hypothesis 1b**), because we expected the per capita grazing rate was expected to be controlled primarily by temperature (Hillebrand, 2009) and periphyton growth rate to be controlled primarily by light and nutrient supply (Dodds, 2007; Lange et al., 2011). To test the net effect of top-down control on periphyton under natural conditions, we conducted a paired large-scale experiment in two mountain streams with strong seasonal changes in light supply and nutrient concentration, in which we exclusively manipulated fish density. We analysed the top-down and bottom-up effects by observing biomass of the benthic grazers and periphyton and by estimating primary production and grazing rates using a model parameterised from laboratory experiments.

2.3 Materials and methods

Experimental site

Two small second-order mountain streams (*Tännichtgrundbach* as control stream and *Gauernitzbach* as treatment stream) served as the experimental sites and sources of material for the laboratory experiments. Both streams are tributaries of the River Elbe in Germany (51°06′46.63′′N, 13°32′45.04′′E; 51°05′12.43′′N, 13°35′55.88′′E). The catchment areas of both streams are dominated by agriculture. In the lower sections, where the study was conducted, both streams flow through a deciduous woodland valley (mainly *Alnus glutinosa*, *Acer* spp., *Quercus* spp.), and because of their narrow width (1.5 – 2.5 m), streams were almost completely shaded by the tree canopy. The streams had similar physical and chemical characteristics and were expected to be sufficiently similar with regard to their benthic community to be used in a comparative experiment (Schmidt et al., 2009b). Although benthic community composition was very similar during phases with similar fish stocks, showing larger differences between pools and riffles within one stream than between the two streams (Schmidt et al., 2009b), some differences could be observed in grazer community composition. While *Rhithrogena semicolorata* larvae (Ephemeroptera: Heptageniidae) had the highest proportion of biomass in both streams, its dominance seemed to be stronger in the treatment stream (Table 2.1, similarity phase). In the control stream, *Electrogena ujhelyii* (Ephemeroptera: Heptageniidae) and *Nemoura cambrica* (Plecoptera: Nemuridae) had higher relative proportions than in the treatment stream (Table 2.1). However, we do not consider these relatively small differences in grazer community composition will have affected the results of this experiment because grazer composition was similar in the two phases in the control stream. Moreover, between-stream differences in physical or chemical factors between the experimental phases, which could have affected the experiment, did not show significant changes (Table 2.2). The only significant difference appeared to be the oxygen saturation between the two experimental phases. However, as the oxygen saturation increased from 84 to 92 % in the reference phase, which is not expected to be critical for fauna, no negative impact on the benthic grazers was expected from any differences between the two streams. On the other hand, nutrient concentrations and other environmental variables did differ between the two streams (Table 2.2). The control stream was characterised by higher nutrient

concentrations, larger discharge and a larger catchment area (Table 2.2, Schmidt et al., 2009b).

Table 2.1 Mean (± SE) biomass proportion (%) of specific grazer species within the total grazer biomass and mean absolute biomass of specific grazer species for the two phases in the control (Con) and treatment (Tre) streams.

	Relative biomass proportion (%)				**Absolute biomass (mg dw m^{-2})**			
	Difference phase		Similarity phase		Difference phase		Similarity phase	
	Con	Tre	Con	Tre	Con	Tre	Con	Tre
Ephem-eroptera:								
Baetis muticus	1.7 ± 0.2	7.4 ± 1.0	4.2 ± 1.6	14.0 ± 2.0	10.5 ± 1.8	74.1 ± 15.3	18.6 ± 9.8	50.2 ± 20.5
Baetis rhodani	24 ± 3.3	9.7 ± 2.3	11 ± 3.3	11.7 ± 2.1	127 ± 22.7	132.5 ± 38.8	50.2 ± 22.2	39.1 ± 15.0
Ecdyonurus sp.	8.2 ± 1.8	4.7 ± 1.0	10 ± 1.8	8.3 ± 1.8	58.5 ± 14.2	80.4 ± 25.1	46.7 ± 18.1	24.0 ± 7.8
E. ujhelyii	7.8 ± 1.7	14 ± 2.5	11 ± 5.6	1.7 ± 0.6	39.6 ± 10.7	103.4 ± 14.9	44.9 ± 22	8.5 ± 3.4
R. semicolorata	1.8 ± 0.8	27.2 ± 4	14 ± 6.7	23.6 ± 6.6	25.4 ± 9.9	454 ± 136.2	156 ± 78.9	142 ± 21.3
Coleoptera:								
Hydraena sp.	7.7 ± 1.6	13 ± 2.9	5.0 ± 3.5	10.1 ± 3.5	28.6 ± 4.4	72.9 ± 11.7	11.2 ± 4.0	17.0 ± 5.5
Plecoptera:								
Leuctra sp.	2.8 ± 0.6	1.7 ± 0.4	2.3 ± 0.8	1.8 ± 0.8	16.7 ± 5.2	7.9 ± 1.3	8.0 ± 3.3	3.1 ± 1.2
Nemoura sp.	6.7 ± 2.3	2.8 ± 0.9	10 ± 4.9	0.9 ± 0.6	91.7 ± 43.0	49.5 ± 15.2	59.4 ± 32.8	6.9 ± 5.11

Monthly nitrate concentrations were not measured throughout the whole experiment, but monthly data for both streams are available for January 2005 to July 2008. Mean nitrate concentrations during that time were 4.6 ± 2.4 mg N L^{-1} (mean ± SD, n = 64) in the experimental stream and 7.3 ± 1.1 mg N L^{-1} (mean ± SD, n = 62) in the control stream. Because these differences did not change between the experimental phases (Table 2.2), we do not expect them to have affected the experimental results, and the nitrogen concentrations in both streams were well above nitrogen levels required for periphyton maximum growth (86 µg N L^{-1}, Rier and Stevenson, 2006).

Influences of agriculture in the catchment areas on water quality might be indicated by the nutrient level. However, we expect possible impacts of land use to be relatively small because of the forest fringe of the stream (100 to 500 m wide). This view is supported by the species-rich benthic invertebrate fauna (125 species excluding chironomids).

Table 2.2 Means (± SE) of environmental factors, the number of sampling occasions (*n*), and the significance levels (*p*) of the changes in the stream treatment differences between the two experimental phases in the control and treatment streams. Italic values indicate significant differences (RIA, randomised intervention analysis).

	Difference phase			**Similarity phase**			**Phase difference**
	Control	Treatment	*n*	Control	Treatment	*n*	*p*
pH	7.8 ± 0.04	8.3 ± 0.03	37	8.0 ± 0.08	8.4 ± 0.03	12	0.13
Electrical conductivity (μS cm^{-1})	523 ± 22	575 ± 13	36	533 ± 22	550 ± 30	13	0.07
Oxygen saturation (%)	84 ± 2	84 ± 2	34	98 ± 2	82 ± 2	12	*0.03*
Discharge (L s^{-1})	56 ± 6	35 ± 4	38	79 ± 12	46 ± 7	9	0.40
Temperature (°C)	7.6 ± 1.1	8.3 ± 1.3	13	8.7 ± 2.1	7.9 ± 2.0	8	0.47
Phospate (μg P L^{-1})	50.7 ± 4.7	36.0 ± 4.5	23	48.1 ± 10	29.2 ± 6.8	8	0.18

Field experiment and sampling

In the field experiment, the influences of benthivorous fish (*Gobio gobio* and *Barbatula barbatula*) stocked simultaneously on the biomasses of invertebrate grazers and periphyton were measured. Small benthivorous fish species rather than drift-feeding trout were chosen because benthivorous fish are expected to have stronger predation impact on benthic invertebrate community than trout (Dahl, 1998b). They often dominate the natural fish community in small European streams and are not as often used as trout in predation experiments. We expect the two fish species to use the full prey spectrum because of their relatively large mouth gape compared to their small body size (gudgeon: mean total length 100.0 ± 24.2 mm, average gape width 6.4 ± 1.7 mm; stone loach mean total length 101.1 ± 30.0 mm, average gape width 5.0 ± 0.5 mm).

To assess the influence of benthivorous fish on benthic grazers and periphyton, the biomass differences were compared between two streams ('treatment stream'-'control stream') during a phase with different fish stock in the two streams (one stream with fish and one without) and one phase of similar fish stock in both streams. A higher or lower mean between-stream difference in the phase with different fish stock (difference phase) compared with the phase with similar fish stock (similarity phase) served as an indicator for fish-related effects. By analysing the differences between the two streams, any temporal variability as well as the effects of factors influencing the two streams in the same way were minimised (Smith et al., 1993).

The large-scale field experiment roughly followed the BACI design (Before-After-Control-Impact, Stewart-Oaten et al., 1986). In contrast to a classical BACI design, in this experiment, fish stock was manipulated in both phases to ensure the desired fish densities. Therefore there is no reference phase in the sense of natural and undisturbed conditions. In addition, the phase with different fish stocks ('difference phase') was analysed first and the 'similarity phase' subsequently. The time schedule was a result of an ongoing long-term experiment on predation effect of benthivorous fish on benthic invertebrates, which required differences in fish stocks between the two streams in 2008 and 2009. We assume this statistical design to be robust because the general design does not necessarily need any natural reference and does not include any directional aspects. However, it supposes that the system returns to the same state every time that the fish biomass is at the same level (resilience), instead of switching to a new state irreversibly. Within the two streams, two consecutive sections, containing approximately 20 and 10 pool-riffle sequences, respectively (400 m and 200 m long), were set up by fish barriers made of a high-grade steel mesh (5-mm mesh size). The barriers were cleaned at least every second week by moving leaf litter and woody debris from the up-stream side of the fence to the down-stream side. The lower section was used as the experimental site and the upper section as a buffer. By including the buffer section possible edge effects on the upper end of the experimental site were minimised (for further explanations see Winkelmann et al., 2011). Before 2006, stone loach and gudgeon inhabited the two lower sections of both streams due to previous experimental stocking. All other fish species sporadically found during electrofishing (*Oncorhynchus mykiss, Cottus gobio*) were removed. In the treatment stream, the two sections and the total stretch

upstream suitable for fish habitation (0.5 km) were made fish-free by four electrofishing campaigns (EFGI 650, Bretschneider Spezialelektronik, Germany) from autumn 2006 to autumn 2007. During the 'difference phase' (January 2008 to October 2009), the control stream was stocked with stone loach and gudgeon while the treatment stream remained quasi fish-free (Table 2.3). During the reference phase (November 2009 to August 2010), both streams were inhabited by benthivorous fish (Table 2.3). The different time spans of difference and similarity phases are due to the assumption that the release of predation pressure from fish (difference phase) might take several grazer generations and therefore several years to manifest itself as differences in grazer biomass, while the release of periphyton from benthic grazers (similarity phase) might take some algae generations, which is possible within few weeks.

Table 2.3 Mean (± SE) fresh mass and densities of benthivorous fish, and the number of sampling occasions (*n*) in the two phases in the control (Con) and treatment (Tre) streams.

	Difference phase			**Similarity phase**		
	Con	Tre	*n*	Con	Tre	*n*
Biomass (g m^{-2})						
Gudgeon	1.01 ± 0.39	0.002 ± 0.002	6	1.76 ± 0.93	2.37 ± 0.89	2
Stone loach	1.19 ± 0.44	0.04 ± 0.03	6	1.27 ± 0.44	1.40 ± 0.54	2
Total	2.20	0.042		3.03	3.77	
Density (Ind m^{-2})						
Gudgeon	0.12 ± 0.051	0.0002 ± 0.0002	6	0.18 ± 0.07	0.29 ± 0.10	2
Stone loach	0.18 ± 0.06	0.003 ± 0.002	6	0.16 ± 0.06	0.20 ± 0.08	2
Total	0.30	0.0032		0.34	0.49	

Fish density in all sections of both streams (plus the upstream reach of the treatment stream during the impact phase) was controlled on seven dates by electrofishing two times on each occasion. Thus, disturbance level was similar between the two streams. All caught individuals were counted and the length of at least 50 individuals was measured. All fish caught in the treatment stream during the

fish-free phase were removed. The streams were allowed to recover for at least three weeks from electrofishing before the next sampling to minimise the influence of the disturbance on benthic invertebrates or periphyton. Any effect of electrofishing would have occurred in both streams and therefore would not have influenced the statistical analysis. During the phase when fish were present in the treatment stream and during the whole experiment in the control stream, stone loach and gudgeon were restocked at least twice every year. Fish stock losses were mainly due to predation by grey herons, winter mortality, occasional emigration during floods and sampling for fish consumption and diet analysis, which were carried out in connection with another study performed at the same site. Fish were obtained from regional streams (Saxony).

The invertebrate community was sampled every 28 days with a Surber sampler (0.12 m^2, 500 μm mesh size). In each stream, three samples were taken on each occasion in a randomly chosen riffle within the respective experimental section. All samples were rinsed over a 500-μm sieve and stored in 80 % ethanol. Periphyton biomass was sampled at least every 28 days (every 14 days in 2008) within the experimental section using the riffle upstream of the one sampled for invertebrates by randomly choosing three stones. It was not possible to use the same riffle for sampling invertebrates and periphyton because the riffle was disturbed during invertebrate sampling. Environmental factors were monitored in both streams at the experimental sites. The water temperature was measured with data loggers in 15-min intervals (Hobo, Bourne, USA). The light supply was estimated using a PAR sensor (Theodor Friedrichs & Co., Schenefeld, Germany) placed on the bank of each stream (June 2009 to June 2010), and measurements were logged every 5 min (Voltvox Maxi, Scantronic, Zorneding, Germany). We decided to give a high temporal resolution priority over spatial resolution of measurements because seasonal shifts were the focus of this experiment. Although light supply could only be measured at one location per stream, it is expected to be representative for the experimental stream section because both banks and the stream itself were fully shaded by canopy. Oxygen saturation, pH value, and electrical conductivity were measured on each sampling occasion in the field using appropriate probes (LF196, pH196, Oxi96, WTW Weinheim, Germany), mostly between 8 and 12 a.m. To determine nutrient concentrations, 100 mL stream water was filtered on site (0.45 μm cellulose acetate) every 28 days and stored cool until laboratory analysis.

Laboratory experiments and sampling

Two sets of laboratory experiments were conducted to estimate the parameters necessary for modelling periphyton grazing and periphyton growth. The effect of temperature and larval development stage on grazing rates was assessed in a grazing experiment while the dependency of periphyton growth on light and phosphate supply was analysed during a periphyton growth experiment.

The grazing experiment was set up as a two-factorial experiment with the factors temperature (4, 10, 16 °C) and larval body size (small, medium, large) representing different developmental stages. The experiment lasted 48 h and was conducted in six black recirculation tanks (40 cm diameter) with a current velocity of 0.04 ± 0.008 m s^{-1} (mean ± SE), a mean depth of 0.14 m, an oxygen saturation of 85.67 ± 1.99 %, and 12/12 h light/dark rhythm. To obtain six replicates for each factor level, three successive experiments were conducted for each larval size. Periphyton grown on unglazed ceramic tiles was used to provide food for the larvae during the experiments, whereas larvae in the storage tank were fed using periphyton-covered stones from the control stream. Grazing rates were estimated using larvae of *R. semicolorata*, one of the dominant grazer species in these streams. Because larval development of this species is highly synchronised, the experiments were conducted in December for small larvae (mean ± SD: 4.7 ± 0.7 mm), in February for medium-sized larvae (6.2 ± 0.7 mm) and in April for large larvae (8.6 ± 1.0 mm). For each experimental date, at least 360 larvae without black wing pads (i.e. not in the last larval stage) were collected in the control stream and their body length was measured. They were stored in a tank with stream temperate water (10 °C) for use in experiments. For each experiment, 20 larvae were placed in each experimental tank and left to acclimatise for 24 h. To calculate the consumption rate for each experimental unit, three individual *R. semicolorata* larvae were sampled from each tank every 4 h over a 24 h period. All larvae were frozen immediately after sampling and stored in liquid nitrogen. Water temperature, pH, oxygen saturation and electrical conductivity were measured at the end of each experiment and seemed similar among all replicates (mean ± SD, pH: 7.5 ± 0.3, n = 54, oxygen saturation: 92.7 ± 4.0, n = 54, electrical conductivity: 479 ± 6 µS cm, n = 54, water temperature: 4 °C: min. 3.5 °C to max. 6.0 °C, 10 °C: min. 8.5 °C to max. 10.0 °C, 16 °C: min. 15.5 °C to max. 16.5 °C).

The periphyton growth experiment was conducted to analyse the dependence of growth rate from the presumed two most limiting environmental factors, light and phosphate. Nitrogen limitation was not expected because limiting concentrations of nitrogen have been shown to range between 0.1 and 0.3 mg N L^{-1} (Rier and Stevenson, 2006), which is at least one order of magnitude lower than mean nitrate concentrations in the studied streams (4.6 and 7.3 mg N L^{-1}). Growth experiments were conducted in two laboratory flumes (1000 L) using stones from the treatment stream at three phosphate concentrations 15, 33 and 130 µg P L^{-1}. To avoid fluctuations of the stream water chemical composition, artificial freshwater developed for cultivation of chlorophytes was used (DIN 38412 Teil 33, 1991). To prevent possible Si limitation due to the expected diatom dominance in the periphyton, 10 mg Si L^{-1} were added. A light gradient with eight intensities from 2 to 275 µmol m^{-2} s^{-1} (mean ± SD, n = 6; 275 ± 18; 165 ± 14, 117 ± 3; 80 ± 4; 63 ± 4; 28 ± 2; 14 ± 1; 2 ± 0.2 µmol m^{-2} s^{-1}), providing a daily sum of 70 to > 12000 mmol m^{-2} day^{-1}, was set up using the length of the two laboratory flumes and 26 plant lamps (Megaman BR0620P, Langenselbold, Germany). The daily light sum of the highest light level (11657 ± 746 mmol m^{-2} day^{-1}) was higher than the maximum light sum measured at the control stream (April 2010: 9326 mmol m^{-2} day^{-1}) and the second lowest light level (586 ± 66 mmol m^{-2} day^{-1}) was similar to the low light supply during summer (July to October 2009: 278 mmol m^{-2} day^{-1}). Growth rates were determined on three stones for each light intensity and phosphorous concentration. In one experiment (33 µg P L^{-1}) only the four highest light levels could be set up for technical reasons. However, because the light dependency of periphyton growth can best be observed at optimal phosphate concentrations, this omission was assumed to be of minor importance. Periphyton was sampled twice on each stone during the experiment: the first sample (start value was taken after two days of acclimatisation) and the second sample (end value) 12 days thereafter by brushing a defined area of the stone with a tooth brush. At least every second day, 100 mL tank water was filtered (0.45 µm cellulose acetate) to determine the phosphate concentration in the filtrate. If necessary, phosphate concentration in the experimental flumes was corrected by adding the appropriate amount of a KH_2PO_4 solution (2 g L^{-1}). Water temperature, pH-value and oxygen saturation were measured at least every second day and did not show any critical values (mean ± SD, water temperature: 17.3 ± 0.9 °C, n = 80, pH: 8.4 ± 0.2, n = 80, oxygen saturation: 100.8 ± 5.0 %, n = 40, electrical conductivity: 566 ± 98 µS cm, n = 40).

Sample processing and laboratory analyses

To determine chlorophyll *a* concentration, periphyton was removed from entire stones (field experiment) or from a defined area of the stones (laboratory experiments) by brushing the stone surface carefully with tap water. The obtained suspension was filtered using glass fibre filters (Sartorius MGF) with a suction pressure not exceeding 0.3 atm. Extraction and fluorimetric/spectrophotometric analysis were done in accordance with Wasmund et al. (2006) and Ritchie (2008). Filters from the field experiment were stored at -20 °C and those from the laboratory experiments were frozen in liquid nitrogen. For analysis, filters were homogenised (Ultra Turrax, IKA, Staufen, Germany), extracted for at least 3 h in buffered 96 % ethanol and measured spectrofluorometrically (field experiment: LS 50B Luminescence spectrometer, Perkin Elmer, Massachusetts, USA) or spectrophotometrically (laboratory experiment: Specord 205, Analytic Jena, Jena, Germany). The methods differed due to the use of a different laboratory for the periphyton growth experiment. Periphyton biomass was estimated as chlorophyll *a* concentration per area of stone surface. The surface area of the stones sampled in the field experiment was determined by carefully wrapping the stones in aluminium foil and weighing the foil after cutting off all protruding folds. The area cleaned from each stone in the growth experiment was measured directly.

All individuals from each benthic sample were identified to the lowest possible taxonomic level and counted. From each taxon in each sample, up to 100 individuals measured to the nearest 0.1 mm. For each taxon that occurred with less than 100 individuals per sample, all individuals of the sample were measured. The individual body mass was calculated as dry mass by using length-weight relationships (Meyer, 1989; Burgherr and Meyer, 1997; Benke et al., 1999).

Gut fullness of the larvae of *R. semicolorata* from the grazing experiment was determined with the gut fluorescence method (Cowan and Peckarsky, 1990) by extracting chlorophyll *a* and phaeophytin from the mortar-ground animals and processing the samples as described for the periphyton samples in the field experiment.

Soluble reactive phosphate (SRP) concentration in the stream water was measured using a spectrophotometrical analysis (Legler et al., 1988, Lambda, Jenoptik, Jena, Germany).

Nitrate concentration was determined using anion-exchange chromatography (ISC90, Dionex) with an 8 mM carbonate per 1 mM hydrogen carbonate solution as mobile phase and an IonPac AS14A separation column (Dionex) as stationary phase.

Data Analysis

A simple model implemented in R (R CoreTeam, 2013) was used to estimate the periphyton accrual rate and the grazing rate of heptageniids and of the total grazer community on a daily basis (Table 2.4). The relative importance of top-down (grazing) and bottom up (growth) processes was assessed from these two rates. The objective of this model was to indicate the temporal change of the potential grazing pressure on periphyton as one of the possible explanations for the patterns observed in nature. The model is not suitable to explain periphyton dynamics in a natural ecosystem because it is very simple, containing only the parameters temperature and body length for the grazers and light supply, phosphate concentration and temperature for periphyton. The model was designed to achieve a gross estimation of grazing rate and accrual rate. Therefore other factors influencing those rates such as fish presence/absence, current velocity, grazer community composition were left out on purpose – not because we assumed that they had no effects but to keep the model as simple as possible. The input variables (Table 2.4) were the biomass of heptageniids or the total biomass of grazers (mg dry weight m^{-2}) and periphyton (µg Chl *a* cm^{-2}), as well as the light and phosphate concentration (light: mmol m^{-2} day^{-1}, phosphate µg P L^{-1}) measured or extrapolated for each time step. To achieve the largest possible generalisation of seasonal pattern and reduce random noise and the effects of between-year variation, as much data as available were used. Therefore values were obtained from a linear interpolation of the mean monthly values observed in the field experiment and during a previous experiment using the same fish stock management but lacking the in-depth periphyton analysis. Biomass values for grazers were averaged for each month of the year from both streams during phases with fish (control stream: October 2004 to August 2011, treatment stream: October 2004 to October 2006 and October 2010 to August 2011) resulting in 5 to 13 values per month. Biomass values for periphyton were treated the same way as biomass values for grazers but sampling began in January 2008.

Table 2.4 Input variables, model variables, parameters and rates of the grazer-periphyton model. The specific daily consumption of the grazers was measured as the amount of total pigments (TP) in the guts due to digestion. It is assumed that the amount of TP approximates the ingested chlorophyll *a* (Chl *a*).

	Abbreviation	Name	Value	Unit
Input variables	GB_1	Grazer biomass (small individuals)		mg m^{-2}
	GB_2	Grazer biomass (large individuals)		mg m^{-2}
	I	Light supply (daily sum)		mmol m^{-2} day^{-1}
	P	Phosphate concentration		µg P L^{-1}
	PB	Periphyton biomass		µg Chl *a* cm^{-2}
	T	Water temperature (daily mean)		°C
Model variables	C	Consumption of grazers		µg Chl *a* cm^{-2} day^{-1}
	PP	Primary production		µg Chl *a* cm^{-2} day^{-1}
Parameters	d	Fitting parameter	0.3166	
	k_p	Half saturation constant of phosphate	16.89	µg L^{-1}
	k_I	Half saturation constant of light	10,490	mmol m^{-2} day^{-1}
	μ_{max}	Maximum accrual rate of periphyton	0.751	day^{-1}
	k	Carrying capacity periphyton	10.25	µg Chl *a* cm^{-2}
	m	Fitting parameter	0.059	
	T_{ex}	Mean temperature in periphyton growth experiments	17	°C
	Q_{10}	Q_{10}-value of periphyton growth	2	
Rates	c_t	Grazing rate (specific daily consumption)		µg TP mg^{-1} dw day^{-1}
	µ	Periphyton accrual rate		day^{-1}

To estimate the mean seasonal change of the environmental factors, all available field measurements of the control stream were used, even if these were measured before the start of the field experiment. Light supply values (June 2009 to June 2010) were calculated from the daily sum of light input. The water temperatures were measured as the daily means for five years (February 2005 to February 2010) and averaged over all measured years for every day of the year. Values for the phosphate concentration were averaged for every month of 8 years of measurements (March 2002 to August 2010) and were linearly interpolated in order to obtain daily values. Data from different years can be used because the seasonal variation was larger than the variation between individual measurements (unpublished data). Consequently, the seasonal variation was captured for the environmental factors. Because, as indicated above, specific grazing rate of large larvae differed from that of small or medium-sized larvae, grazer biomass and specific grazing rate were calculated for the two size classes separately and summed up later (Table 2.4). For the calculation of total grazer biomass, the mean biomass of each species for every sampling occasion was corrected by a factor related to the food preferences of the local fauna as listed in Schmedtje and Colling (1996) and Tachet et al. (2002). For example the biomass of a grazer species feeding 80 % on periphyton was multiplied by 0.8. Therefore 'grazer biomass' gives the functional biomass of the grazers. Several species in the group described as grazers (Schmedtje and Colling, 1996) were excluded from the analysis because stable isotope analysis did not show their use of periphyton as a food source (Limnephilidae, *Gammarus fossarum*, *G. pulex*, *Isoperla grammatica*) in the streams studied here (unpublished data). In addition, *Ephemera danica* was excluded because this species burrows within the substratum, feeds on very fine sediment and does not use periphyton from stones (personal observations).

To parameterise the model for periphyton accrual rate (μ), the parameters half saturation constant (for phosphate k_P and light supply k_I), and maximum growth rate (μ_{max}) were calculated using a Michaelis-Menten relationship (SigmaPlot, Version 11, Systat Software Inc., Erkrath, Germany) and data from the periphyton growth experiments. To correct the periphyton accrual rate for any seasonal temperature variation, a Q_{10} value of 2 (Table 2.4) was used in accordance with Bothwell (1988) and Hawes (1993).

The calculation of the grazing rate was based on the consumption model of Elliott and Persson (1978). As proposed in Madon and Culver (1993), the exponential evacuation rate was derived from the maximum rate of decline in gut fullness, measured in the grazing experiments because evacuation rates derived from own laboratory experiments were generally lower than the values derived from the field observations probably due to a reduction of gut evacuation during starvation. A general gut evacuation rate was estimated by combining data from all experimental runs and fitting them to an exponential relationship (R Core Team 2013). Then, the consumption model was applied to the single data sets, gained from each of the six tanks and the resulting six consumption rates averaged for each treatment. To test the fish effects on grazer and periphyton biomass, a randomised intervention analysis (RIA, Carpenter et al., 1989) was implemented using the software R (R Core Team 2013). Thereby, the probability that the observed mean difference between the two experimental phases (similarity phase, difference phase) could be random was estimated using a randomisation test and all differences with a probability < 5 % were assumed to be effects of the experimental manipulation. Values for grazer and periphyton biomass were calculated as difference between treatment stream and control stream on each sampling occasion to account for seasonal changes and to reduce autocorrelation of the biomass.

Large and small grazers were used as separate variables because the food intake of the large larvae individuals was significantly reduced. Small individuals were calculated to be < 95 % of the adult size (*Baetis muticus* < 5.0; *B. rhodani* < 6.0; *Capnia bifrons* < 6.9, *Ecdyonurus* sp. < 9.8; *Electrogena ujhelyii* < 8.4, *Leuctra* sp. < 6.9, *Nemoura cambrica* < 5.4, *Protonemura intricata* < 5.7, *Rhithrogena semicolorata* < 8.5, *Seratella ignita* < 4.8 mm).

Following equations were used in the dynamic model:

$$\mu = \left(\mu_{max} * \frac{I}{I+k_I} * \frac{P}{P+k_P}\right) * Q_{10}^{\frac{T-T_{ex}}{10}} \quad \textbf{(2.1)}$$

$$PP = \mu * PB * (1 - PB/k) \quad \textbf{(2.2)}$$

$$c_t = d + e^{(m*T)} \quad \textbf{(2.3)}$$

$$C = (c_t * GB_1/10000) + (c_t * GB_2/10000) \quad \textbf{(2.4)}$$

Equation (2.1) calculates the periphyton net accrual rate for each day of the year according to water temperature, light supply and phosphate concentration. From that rate, the daily primary production is calculated (Equation 2.2) using the field measured values of periphyton biomass and a limitation term, describing growth limitation near the carrying capacity. Equation (2.3) represents the fitted function of the specific periphyton consumption rate by grazers in dependence from water temperature and is used to calculate a specific daily consumption rate. In equation (2.4) a total consumption rate by grazers is calculated using field values of grazer biomass, split into two size classes. The factor 10000 is a conversion factor from m^2 (grazer biomass) to cm^2 (Chl *a* concentration).

2.4 Results

Large-scale evidence of a tri-trophic cascade

A three-level trophic cascade was observed from fish via grazers to periphyton with benthivorous fish indirectly increasing mean annual periphyton biomass. Following restocking in the formerly fish-free stream, an 87 % decrease of mean annual benthic grazer biomass and a 25 % increase of mean annual periphyton biomass relative to the continuously fish-stocked stream was observed (Figure 2.1). In addition, the biomass of grazer and periphyton differed more between the streams in the similarity phase then in the difference phase with fish. The between-stream difference of total grazer biomass changed significantly between the difference phase and similarity phase ($p = 0.045$, similarity phase: $n = 9$; difference phase $n = 23$, randomisation test). In the difference phase, grazer biomass in the control stream and the treatment stream were very similar, as indicated by the low mean difference between the two streams (Figure 2.1). In the similarity phase, when fish were restocked in the treatment stream, the between-stream difference became negative, indicating a lower grazer biomass in the treatment stream in the presence of fish (Figure 2.1). Grazer biomass in the two streams was dominated by mayflies throughout the experimental phases (Table 2.1). The difference of periphyton biomass between the streams increased significantly in the similarity phase ($p = 0.030$, similarity phase: $n = 11$; difference phase $n = 38$, randomisation test) indicating that periphyton biomass increased in the treatment stream relative to the control stream when fish were restocked (similarity phase, Figure 2.1).

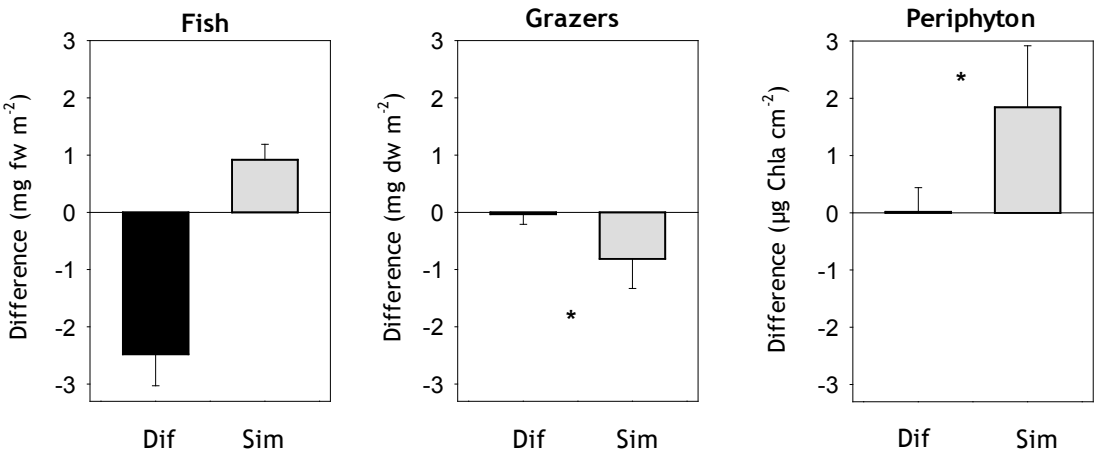

Figure 2.1 Differences (means ± SE over all seasons) of fish biomass (n = 6/2), grazer biomass (n = 23/9) and periphyton biomass (n = 38/11) between the streams during the difference (Dif) and similarity (Sim) phases, calculated as $Biomass_{\text{treatment stream}} - Biomass_{\text{control stream}}$. Significant differences are indicated by *.

Laboratory experiments and model parameterisation

The rate of primary production closely followed Michaelis-Menten kinetics and indicated light and phosphate limitation (Figure 2.2, Table 2.5 and Table 2.6). The half saturation constants for light were shown to depend on the phosphate concentration and *vice versa* (Table 2.5 and Table 2.6), which suggests a possible colimitation of both resources at low phosphate concentrations. The light and phosphate supply levels used in the laboratory experiments covered the range measured in the field experiments. Because the light limitation was much stronger than the phosphate limitation, no k_P values could be estimated for lower light levels (Table 2.6).

To estimate the grazing rate, its dependence on water temperature and grazer body length was analysed using the grazer *Rhithrogena semicolorata* as model organism. Grazing rate increased with water temperature for small and medium-sized larvae of *R. semicolorata* (Figure 2.3) and model parameters were estimated accordingly (d = 0.317, m = 0.059, Equation 3, Table 2.4). Large larvae showed an extreme consumption reduction of 91.5 % compared with the mean feeding rate of small and medium larvae (Figure 2.3), which was accounted for in the model by a 90 % reduction in the grazing rate for all larvae > 95 % of the mean size at emergence.

We extrapolated this reduction to other mayfly species because we assume it to be a general pattern caused by larval development in preparation of maturation. To implement this reduction in feeding rates for other grazing mayfly species, species-specific thresholds were estimated, which equalled 95 % of the mean body mass at emergence for each species and paralleled the differences in grazer size at emergence.

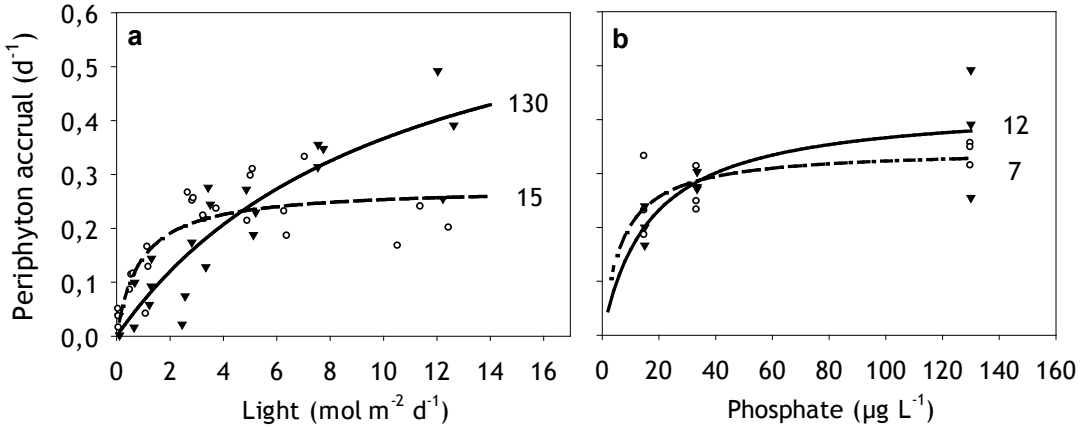

Figure 2.2 Periphyton growth rate dependence on the gradient of (a) light and (b) phosphate supply and the fit of the values to the Michaelis-Menten relationship (n = 3 for each supply level). The numbers in the panels indicate the phosphorus concentration (a, µg L^{-1}) or the light supply (b, mol m^{-2} day^{-1}) at which the relationship was observed.

Table 2.5 Dependence of the half saturation constant of light-limited periphyton growth rate on the phosphate concentration measured in laboratory experiments (eight light levels for each phosphate concentration, three stones for each level).

P concentration (µg L^{-1})	k_I (M m^{-2} day^{-1})	μ_{max}	r^2
130	10.5	0.75	0.809
33	0.9	0.31	0.235
15	0.9	0.28	0.703

Table 2.6 Dependence of the half saturation constant of phosphorus-limited periphyton growth rate on the light supply measured in laboratory experiments (three phosphate concentrations for each light level, three stones for each level).

Light supply (μM m^{-2} day^{-1})	k_P (μg L^{-1})	μ_{max}	r^2
11.7	16.9	0.43	0.599
6.9	6.9	0.35	0.390

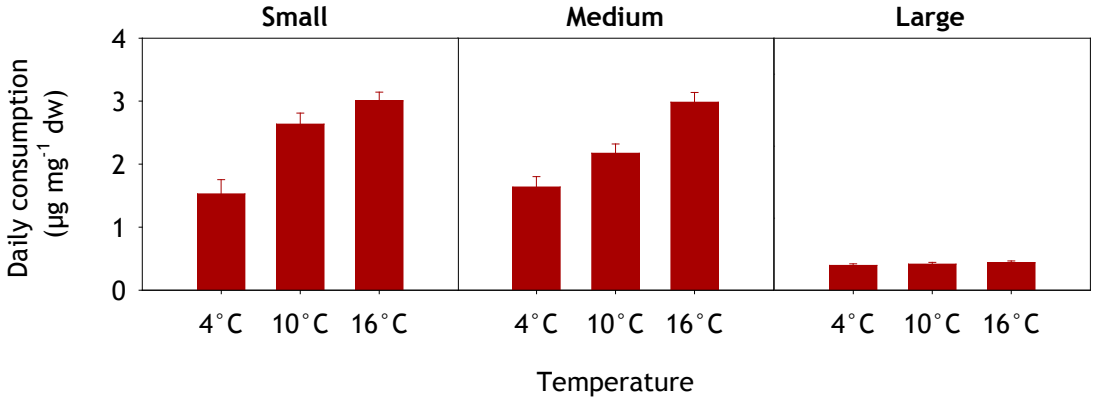

Figure 2.3 Daily consumption (mean + SE, n = 6) by small, medium sized and large larvae of the grazer *R. semicolorata* at three different water temperatures (TP: total pigments). The consumption rate was estimated using six consecutive samples over 24 h for six replicates (36 samples per temperature and size).

Seasonal changes in bottom-up control of periphyton

Primary production in the streams seemed to be light-limited during most of the year. Only during a short period in spring (March – April) did the mean daily light input in the field exceed the half saturation constant (k_I) measured in the laboratory experiments (Figure 2.4a). Nutrient limitation seemed to be less important because phosphate supply exceeded the half saturation constant (k_P) over the whole year. There were only very few days in April, with especially high light supply, when k_P ranged near the phosphate concentrations measured in the field (Figure 2.4b). The modelled periphyton accrual rate showed a peak in spring, in concurrence with the higher light supply during this time of year (Figure 2.4c). The mean periphyton biomass measured in the stream followed a similar pattern (Figure 2.4d). This

indicates that the calculated daily rates based on parameters both from stream and laboratory, described patterns in the stream reasonably well.

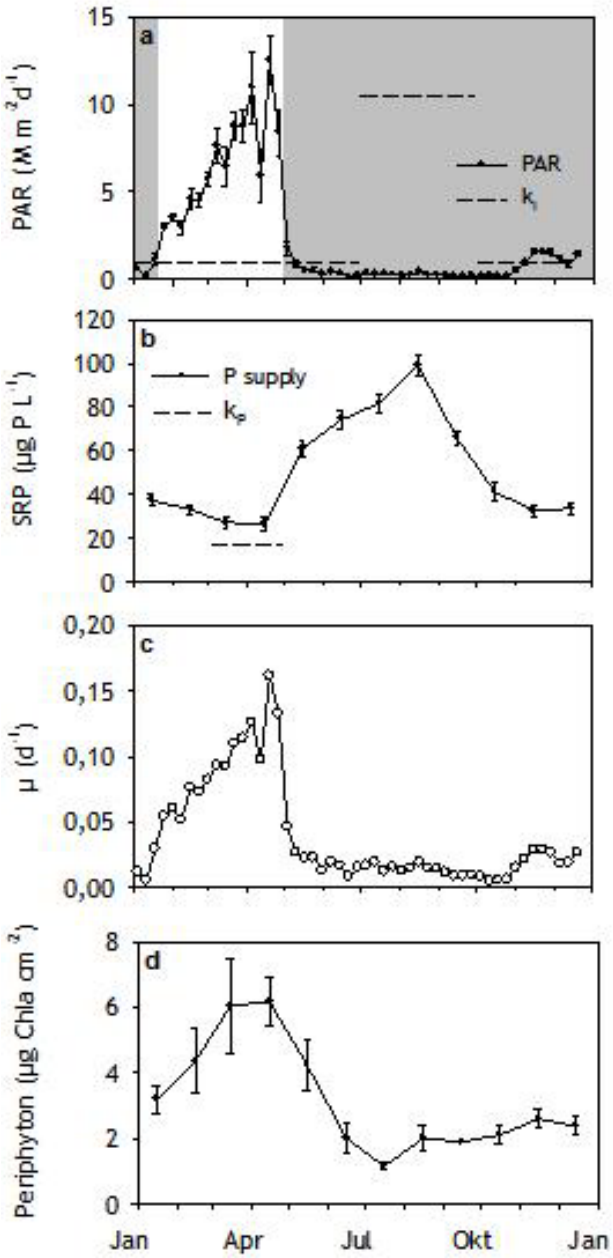

Figure 2.4 (a) Daily light supply measured as photosynthetic active radiation (PAR), (b) phosphate supply, (c) periphyton accrual rate (model estimation) and (d) periphyton biomass (means ± SE) in the control stream. Broken lines indicate estimated half saturation constants and grey area indicates the time span with light limitation.

Seasonal changes in top-down control of periphyton

Model estimations indicate that the magnitude of top-down control on periphyton varied seasonally. The grazing rate in the stream can be expected to range between the estimated grazing rate of heptageniid mayflies, comprising in average 33 - 35 % of the total grazer biomass in the two streams, and the estimated grazing rate of the total grazer community.

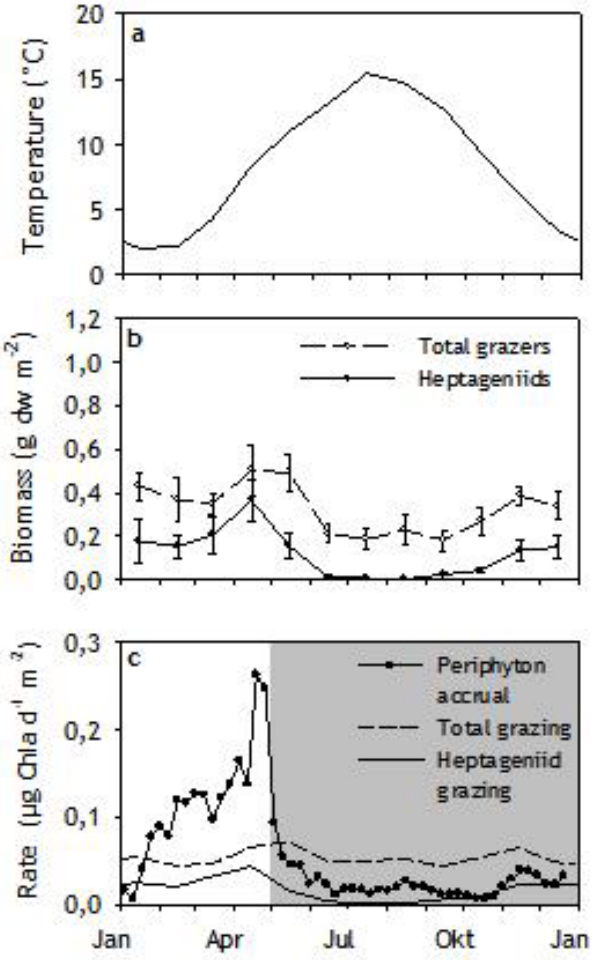

Figure 2.5 (a) Mean water temperature used in the model estimation, (b) biomass of heptageniids and the total grazer community (mean ± SE) used for the estimations and (c) model estimation of the periphyton accrual rate and the rate of periphyton consumption by heptageniids and by the total grazer community. Grey area indicates top-down control on periphyton.

For both estimations, the grazing rate obtained in laboratory experiments for *R. semicolorata* was extrapolated using the biomass of heptageniids or total grazer community. The comparison of the estimate of the grazing rate with the estimate of the periphyton accrual rate indicates that top-down control is strong from June to January and low from February to April. This seasonal shift is caused by a high rate of primary production during the springtime and by a low rate of primary production from summer to early winter (Figure 2.5c). In contrast, the grazing rate was estimated to be relatively constant over the year in spite of distinct seasonal changes in temperature (Figure 2.5a) and biomass of heptageniids or total grazer community (Figure 2.5b). Consequently, in spring benthic grazers seem to consume only a part of the total primary production (heptageniids: 11 %, total grazers 42 % of mean of daily primary production from March to April), which is expected to result in a positive periphyton net growth rate. The grazing rate in autumn and early winter seems to be at least of the same order of magnitude as the periphyton accrual rate, indicating that primary production might be completely consumed by the grazers (Figure 2.5c), which consequently might even result in a negative net accrual rate of periphyton.

2.5 Discussion

We hypothesised that periphyton biomass in streams can be top-down controlled by benthivorous fish (**Hypothesis 1a**). In addition, we expected that seasonal shifts between top-down and bottom-up control in temporally shaded streams might weaken the top-down impact on periphyton (**Hypothesis 1b**). Strong seasonal shifts are expected to result in time periods when the limitation of primary production would be very weak due to excessive resource supply, whereas at other times primary production is strongly resource-limited. Only during periods of strong resource limitation can periphyton reduction by grazing be expected without the losses being compensated.

The results of the field experiment support our first hypothesis (**Hypothesis 1a**) and indicate a trophic cascade induced by benthivorous fish. The averaged between-stream differences in grazer biomass and periphyton biomass changed significantly after fish were restocked in the experimental stream. We expected that the biomass of grazers and periphyton would be very similar between the two streams in the similarity phase and differ in the fish phase, but we actually observed the

contrary. Periphyton biomass during the phase of similar fish stock was consistently lower in the control stream, and grazer biomass was consistently higher. We explain this somewhat unexpected result with inherent differences in the environmental characteristics between the two streams. Although the two streams were compared in previous studies and found to be reasonable similar, the control stream has a somewhat larger catchment area, a slightly larger discharge and higher phosphate concentrations (Schmidt et al., 2009b), which might lead to different standing stocks of periphyton and grazers in the two streams at a similar predation pressure. We do not consider that these initial between-stream differences weaken our results because the fact remains that the absence of benthivorous fish in the experimental stream increased grazer biomass and reduced periphyton biomass relative to the control stream, which indicates the existence of the three-level trophic cascade.

While changes in fish stock often produce changes in food web structure in aquatic food-web structure (Eby et al., 2006), the importance of top-down control in stream ecosystems is still debated because most examples of dramatic changes of food-web structure following changes in fish stock come from lake ecosystems (Scavia et al., 1986; Carpenter et al., 1996; Benndorf et al., 2002). Moreover, although there are some large-scale studies documenting the effect of changes in fish stock on benthic community (McIntosh et al., 2005; Meissner and Muotka, 2006) or direct effects of fish or vertebrate grazers on periphyton biomass (McIntosh et al., 2005; Taylor et al., 2006; Whiles et al., 2013), there are only two reports of three-level trophic cascades from fish via benthic invertebrates on periphyton (Huryn, 1998; Buria et al., 2010). In these two studies, the presence of fish increased periphyton biomass via a reduction of benthic invertebrate grazers similar to our findings. But in contrast to this study, the fish predators were in both cases newly introduced exotic species (rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* (Huryn, 1998; Buria et al., 2010). To our knowledge, there is no large-scale study on the top-down impact of benthivorous fish on periphyton. However, because the impact of benthivorous fish seems to be even larger that that of drift-feeding trout (Dahl and Greenberg, 1996), the effects shown in this study seem plausible. Other studies of experimental or disease-driven species removals or invasion-related species addition point in the same direction and document ecosystem-wide effects on the adjacent trophic level such as the increase of periphyton induced by a loss of detritivorous fish species (Taylor et al., 2006) and tadpoles (Whiles et al., 2013).

It could be argued that so few records of trophic cascades exist in streams in contrast to lake ecosystems because they are very unlikely to occur. This could be explained by general structural differences of food webs between lakes and streams such as the significantly higher degree of omnivory in streams (Digel et al., 2011), which might decrease the probability of the occurrence of trophic cascades. However, other factors such as nutrient and light supply as well as their seasonal changes could be responsible for the attenuation of trophic interactions within the food web. Indeed, a combination of top-down and bottom-up regulation of periphyton was observed in New Zealand stream ecosystems invaded by exotic trout (Huryn, 1998). The effect of grazers seems to be compensated by enhanced periphyton accrual when the periphyton biomass is not resource-limited, for instance due to anthropogenic nutrient enrichment (Dube et al., 1997).

Similar observations were made in lakes, where reductions in the planktivorous fish stock resulted in low phytoplankton biomasses only for phosphate concentrations between certain threshold values (Carney, 1990; Jeppesen et al., 2003). Under the condition of strong phosphate limitation, the grazer biomass was resource-controlled in these lake studies. If the phosphate supply was too high, the grazer-phytoplankton interaction was decoupled due to the dominance of non-ingestible phytoplankton species (Benndorf et al., 2002). This parallels the results of our study, which also indicates control of periphyton biomass by a combination of resource availability and grazing pressure, which was in this case influenced by seasonal changes of environmental factors and grazer biomass due to phenological pattern (emergence).

Because the data of the field experiment did not allow testing for seasonal changes in top-down control, we used a model to estimate the grazing pressure over time. The model estimation indicates a stronger grazing pressure during summer and autumn than during spring supporting our second hypothesis (**Hypothesis 1b**). This can be explained with a more or less constant grazing rate, which cannot counter the seasonal changes in periphyton as fast as periphyton profits from an increasing light supply. This temporal asymmetry of grazing rate and primary production can be linked to the large differences of generation length. While the generation length is only a few days in most benthic algae (John et al., 2002), it is mostly one year for grazers (Bauernfeind and Humpesch, 2001). Consequently, the periphyton biomass can react quickly to seasonal shifts in resource availability,

whereas grazer biomass cannot. It has been predicted before that such time lags can prevent consumers from regulating their resources (Arditi and Ginzburg, 1989; Power, 1992). Therefore we assume that this fact weakens the trophic cascade.

Based on the experimental results and model estimates, we assume that the grazer density was limited by a phase of minimum primary production around its reproduction time (July to October). This resulted in a grazer biomass, which was too low to be able to diminish effectively the high periphyton biomass later in the same generation (next spring). Specific grazing rate (C_t) was determined largely by grazer biomass and to a lesser extent by water temperature and larval stage, as laboratory experiments with one grazer species indicated. Primary production, on the other hand, was shown to be light-limited during the summer and autumn because of dense deciduous vegetation, which was indicated by light supply below the periphyton half saturation constant during this time. Consequently, during this time, primary production was bottom-up controlled by light-limitation and strongly top-down controlled by grazing.

Fish-induced changes of periphyton biomass in this study were estimated to amount to about 25 % of the annual mean. A stronger top-down control by fish might be expected in streams, which are not shaded by deciduous forest and therefore do not experience such strong seasonal light supply shifts. The resulting more stable periphyton biomass would be expected to allow the development of a high grazer biomass and the temporally more constant use of primary production, which was observed in other large-scale studies (Katano et al., 2007; Buria et al., 2010).

The high significance of light supply on periphyton-grazer interactions seems not to be a general pattern. Neither Buria et al. (2010) nor Erös et al. (2012) found any clear bottom-up effects of changing light intensity in temporally shaded streams. However, the very low phosphate concentration in these streams suggests that periphyton accrual rate was instead limited by the phosphate. If periphyton accrual rate is light-limited, as in our study, seasonal changes in light supply due to leaf emergence on stream side vegetation can cascade upwards through the stream food web (Hill et al., 2001, this study). This is an important result because seasonal shifts in environmental factors and any resulting seasonal changes of the associated biotic interactions have previously received little attention in stream ecology. In most other field experiments, seasonal changes are excluded either due to the use of short experiments or by the averaging of serial measurements. If seasonal changes

are included, a strong variation in the intensity of top-down control intensity can be observed (Rosemond et al., 2000; Katano et al., 2007).

An interesting side aspect of this study was the finding that large *Rhithrogena semicolorata* larvae showed a reduction of 90 % in their consumption. Although the larvae used in the experiments did not have black wing pads - which indicates imminent metamorphosis - a reduction in feeding activity caused by an advanced larval development stage seems possible because the mean body length of the large *R. semicolorata* larvae used in the experiment was similar to that of the *R. semicolorata* imagines in the field (8.6 ± 1.0 mm vs. 9.0 ± 1.1 mm, mean ± SD). The consequence of this finding for stream ecosystems, should it apply to mayfly larvae in general, could be a lower grazing rate in spring than might be expected due to the high grazer biomass. However, a comparison of model estimations with and without this reduced consumption indicates a slightly higher grazing pressure without this reduction, which would not change the general pattern of too low grazing pressure to control the periphyton spring peak.

We stress that our model estimations of grazing rate and periphyton accrual rate have to be treated with caution. The model is not suitable for explaining periphyton dynamic in an ecosystem because it is very simple and contains only the parameters temperature and developmental stage for the grazers and light supply, phosphate concentration and temperature for periphyton. The model results have to be interpreted based on the assumption that those are the parameters most influential in the studied systems and further investigation might reveal other influential factors. However, we still believe that the model is suitable for the aim of this investigation indicating theoretically the temporal change of the grazing pressure on periphyton, which was one of the possible explanations for the patterns observed in nature. We expect the realistic value of community grazing rate to range between our minimal estimation including only the biomass of heptageniid grazers and our rough extrapolation to the total grazer community. The latter value is to be treated with caution, because the grazing rate of one heptageniid species is used for this extrapolation, ignoring possible species-specific differences in grazing rate. However, even the estimation for heptageniids shows that grazing rates were roughly in the same order of magnitude as periphyton accrual rates during autumn and early winter, indicating a strong top-down regulation of periphyton biomass.

We are confident that the statistical analysis of the field results indeed shows the existence of a trophic cascade. In contrast to classical BACI-type experiments, our study was not an analysis of an anthropogenic impact assumed to change the 'natural state' of the ecosystem. Instead we compared two alternative states: 'low fish predation' and 'high fish predation'. It might be argued that the experimental design using a phase with similar fish stock afterwards and not before the phase with different fish stocks could affect the results. However, we assumed that the release from fish predation pressure might take longer to show effects than the release of periphyton from benthic grazing due to the differences in generation time of invertebrates and algae. Therefore the fish free situation in the experimental stream was established in autumn 2006 allowing at least one grazer generation to pass before the start of the actual sampling (January 2008) which then occurred over nearly two years, allowing another two generations of grazers to develop without fish predation. We assume that a strong recovering pattern that might have occurred between autumn 2006 and January 2008 can thereby be largely excluded from our data set.

3 Effects of fish predation on grazer-periphyton interaction

Effects of benthic grazers on periphyton community composition in different manipulated streams

3.1 Abstract

To assess the strength of trophic cascades in moderately eutrophied streams, we conducted a paired long-term field experiment at the ecosystem level to quantify cascading effects of benthivorous fish (stone loach: *Barbatula barbatula* and gudgeon: *Gobio gobio*) via benthic invertebrate grazers (Ephemeroptera: *Baetis rhodani*, *Electrogena ujhelyii*, *Rhithrogena semicolorata*, *Ecdyonurus* sp.) on periphyton community composition.

The experimental design followed the before-after-control-impact approach (BACI), manipulating the density of benthivorous fish in two mountain streams. The initial phase was characterised by different fish densities in the streams ('difference phase', i.e. control stream with fish, treatment stream without fish), while during the subsequent 'similarity phase' both streams had similar fish densities.

The aims of this study were to examine if (1) the presence of benthivorous fish would reduce grazer biomass and thus increase periphyton biomass, (2) any fish-induced changes in grazer biomass could alter the periphyton community composition due to selective feeding on certain benthic algal groups, and (3) fish presence would impact feeding selectivity of benthic grazers.

Our results indicated a classical trophic cascade: the presence of benthivorous fish (i) induced a reduction of the total grazer biomass and thus indirectly increased the periphyton biomass, (ii) affected the periphyton community composition as the relative portion of total diatoms significantly increased, and (iii) significantly reduced stalk/tube forming diatoms due to the increase in biomass of grazer species feeding on this algal group. There were no indications, however, that the presence of benthivorous fish affected dietary preferences of benthic grazers.

We conclude that detailed examinations of the benthic grazing process, including the consideration of feeding traits of particular benthic grazer communities,

are necessary to understand the strength of trophic interactions between grazers and their primary food source, periphyton. Otherwise, it will be difficult to predict the potential of benthivorous fish to impact the composition of benthic algal communities via benthic grazers (top-down trophic cascade).

3.2 Introduction

Periphyton and benthic invertebrate grazers constitute crucial food web components in stream and river ecosystems. It is thus critical to understand the complex processes that structure their communities. While the effects of fish predation on benthic grazers (e.g. McIntosh, 1995; Herbst et al., 2009; Winkelmann et al., 2011) and grazer effects on periphyton biomass are well studied (e.g. Feminella and Hawkins, 1995; Liess and Hillebrand, 2004), only few studies dealt with indirect effects of predatory fish on periphyton community composition (Buria et al., 2010; Kurle and Cardinale, 2011; Alvarez and Peckarsky, 2014). These studies exclusively used different species of trout or trout chemical cues, however, there is still a knowledge gap regarding the cascading effects of benthivorous fish on the assemblage composition of benthic algae in stream ecosystems.

Cascading trophic effects have been described for many aquatic ecosystems (e.g. Power et al., 1985; Carpenter et al., 1987; Strong, 1992; Schmitz et al., 2004). In particular, lotic fish as top predators are known to control lower trophic levels via trophic cascades indirectly due to their feeding behaviour and the avoidance behaviour of their prey (e.g. Power, 1990a; McIntosh and Townsend, 1996; Dahl, 1998a; Winkelmann et al., 2014). These indirect interactions have often the potential to mask and overwrite the direct effects and thus, make it difficult to interpret or predict community and food-web dynamics (e.g. Menge, 1995; Abrams et al., 1996; McCann et al., 1998). Although less frequently studied, trophic cascades from fish via benthic grazers to periphyton have been observed for stream ecosystems (e.g. McIntosh and Townsend, 1996; Buria et al., 2010; Winkelmann et al., 2014). Those studies, however, focused on quantitative analysing, not considering the periphyton community composition and the probable processes controlling it.

Predators can have both lethal and sublethal effects on prey communities, whereat sublethal effects can even be stronger than lethal one due to alterations in prey behaviour, morphology or life history traits (Allan, 1981; Gerking, 1994). Both lethal and sublethal predation effects reduce the overall grazing rate of benthic

grazers, as prey organisms use behaviour adaptations to reduce the encounter rates with their predators. Typical avoidance behaviours of benthic invertebrates include shifts in habitat use, change in activity level, increased drift activity and alterations in foraging behaviour (e.g. Sih, 1980; Gilliam and Fraser, 1987; Peckarsky et al., 1993; McIntosh and Townsend, 1994; Tikkanen et al., 1994; Huhta et al., 2000; Winkelmann et al., 2008). It seems therefore at least possible that the invertebrate grazers may change their feeding selectivity in response to fish presence. This means that mayfly grazers possibly use less favoured food items to avoid fish encounters.

On the other hand, benthic grazers are able to alter periphyton assemblages by affecting not only biomass and primary production but also by affecting taxonomic composition and physiognomy by preferentially consuming specific algal growth forms or by disturbing specific algal taxa (e.g. Feminella and Hawkins 1995, Liess and Hillebrand 2004, Steinman 1996). Attached algal growth forms, for example, seem to be more resistant to grazing than those with stalked or erected physiognomies (Sumner and McIntire, 1982). Consequently, growth form, attachment ability and size are integral traits for algal species to withstand the removal by grazers (e.g. Gregory, 1983; Jacoby, 1987). Due to the considerable natural variability, previous studies that dealt with grazer-periphyton interactions have largely focused on the consequences for algal communities (e.g. Colletti et al., 1987; Sturt et al., 2011; Peters and Traunspurger, 2012). Surprisingly little is known, however, about the dietary selection and preference of benthic grazer species, especially with respect to different levels of predation risk within a long-term field experiment at the ecosystem level.

So far, the majority of grazer-periphyton studies were performed on small temporal and spatial scales in artificial streams (e.g. DeNicola et al., 1990; McCormick, 1994; Wellnitz and Ward, 2000; Villanueva and Modenutti, 2004b). Yet, to properly evaluate interspecific trophic interactions (e.g. fish-grazer-periphyton interactions) experiments should be conducted at larger scales (e.g. riffle, reach, stream) rather than at smaller scales (e.g. microhabitat; Doi and Katano, 2008). Accordingly, a three-year, ecosystem-scale before-after-control-impact (BACI, Stewart-Oaten et al., 1986) experiment was conducted in two small streams where fish densities were experimentally manipulated. To quantify the indirect effects of benthivorous fish (presence/absence) on periphyton, we measured the biomass of benthic grazers and

periphyton, analysed grazers gut contents and their dietary preferences, and the natural assemblages of algal resources in both streams during a complete mayfly generation (dominant grazers in the system). We hypothesised that the presence of benthivorous fish will reduce grazer biomass resulting in increased periphyton biomass (**Hypothesis 1a**), can induce an alteration in periphyton community composition due to biomass changes of grazer species which prefer certain algal food items (**Hypothesis 2a**), and will change the feeding selectivity of benthic grazers (**Hypothesis 2b**).

3.3 Materials and methods

Study site

The experimental manipulations were carried out in two small second order mountain streams (*Tännichtgrundbach* as control stream and *Gauernitzbach* as treatment stream) draining into the River Elbe (Saxony, Germany, 51°06´N, 13°32'E, 120 m above sea level). Catchments of both streams were dominated by agriculture. The experimental reaches were located in deciduous woodland valleys (mainly *Acer* spp., *Alnus glutinosa*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robus*). Both streams are characterised by distinctive pool-riffle sequences and high substrate diversity.

Table 3.1 Environmental factors (means ± 1 SE) and number of measurements (*n*) in the control and treatment streams during the years 2008 and 2009. Italic values indicate significant differences (Welch two sample t-test).

	Control	***n***	**Treatment**	***n***	***p***
Temperature (°C)	9.5 ± 0.6	38	9.2 ± 0.6	37	0.79
Oxygen saturation (%)	90.2 ± 2.1	36	90.6± 2.1	35	0.89
Oxygen concentration (mg L^{-1})	9.5 ± 0.3	36	9.6 ± 0.3	35	0.78
Electrical conductivity (µS cm^{-1})	578.1 ± 20.1	37	639.1 ± 18.0	37	0.05
Discharge (L s^{-1})	64.5 ± 8.5	39	38.5 ± 5.3	39	*0.02*
pH	7.9 ± 0.1	38	8,2 ± 0.0	39	*0.002*
Phosphate (µg P L^{-1})	49.9 ± 4.0	24	35.1 ± 3.9	24	*0.02*
Nitrate nitrogen	7.3 ± 0.2	8	6.3 ± 0.5	8	0.07

With respect to size, morphology and species composition both streams were relatively similar (Schmidt et al., 2009b). Differences in environmental conditions between the streams (Table 3.1), especially discharge, pH and phosphate concentration were accounted for by the experimental design.

Study species

We used two benthivorous fish species, gudgeon (*Gobio gobio* L., Cyprinidae) and stone loach (*Barbatula barbatula* (L.), Cobitidae), as vertebrate predators during the experiments. Both fish species are similar in size and ecology and both feed on mayfly larvae. Total length of the gudgeon and stone loach were 96.0 ± 15.1 mm (mean ± SD, n = 401) and 100.0 ± 27.5 mm (mean ± SD, n = 464), respectively with no significant differences between streams (for details in fish ecology see Worischka et al., 2012).

Table 3.2 Mean functional biomass, in dry weight and mean density (± 1 SE, n = 33) of studied grazer species in the control and treatment streams during a complete mayfly generation (September 2008 to July 2009). The biomass of each grazer was corrected by the food preference factor (given in brackets) as listed in Schmedtje and Colling (1996) and Tachet et al. (2002). For example, factor 8 indicates the preference for feeding 80 % on periphyton. Italic values indicate significant differences (Welch two sample t-test).

Species	**Biomass (mg m^{-2})**			**Density (Ind m^{-2})**		
	Control	Treatment	p	Control	Treatment	p
B. rhodani (8)	51.8±6.7	39.8±10.1	0.57	295.2±35.4	214.5±29.2	0.32
Ecdyonurus sp. (5)	24.3±3.9	39.4±7.6	0.45	31.8±6.2	65.0±8.4	0.09
E. ujhelyii (5)	11.3±1.8	20.1±2.6	0.13	66.4±11.0	170.9±31.1	0.09
R. semicolorata (10)	9.6±2.1	239.0±46.2	*0.02*	7.8±0.7	649.8±74.8	*0.002*

Our analyses focused on four dominant grazer species of the two streams: *Baetis rhodani*, *Ecdyonurus* sp., *Electrogena ujhelyii* and *Rhithrogena semicolorata* (Table 3.2), which are common mayfly species (Ephemeroptera) in similar ecosystems throughout Central Europe.

Over an extended period these species represented together 40 ± 9 % (mean 2002 to 2010 ± SD, n = 297) of total grazer biomass in the treatment stream and 44 ± 10 % (mean 2002 to 2010 ± SD, n = 297) of total grazer biomass in the control stream. All four species belong to the functional feeding group of scrapers (Cummins and Klug, 1979) using periphyton as a food source to a great extent (at least 50 %) (Schmedtje and Colling, 1996; Tachet et al., 2000) and prefer riffle habitats of running waters (Bauernfeind and Humpesch, 2001; Elliott and Humpesch, 2010).

Study design

Fish densities were manipulated and controlled in each stream as part of a larger experiment (for details see Winkelmann et al., 2011). For this purpose the study reaches of each stream were separated into two consecutive sections divided by fish barriers (high-grade steel mesh, mesh size 5 mm). The downstream sections (400 m) of both streams were used as experimental reaches, and the upper sections (200 m) were buffer reaches to prevent benthic invertebrates from upstream sites entering the experimental reaches. To avoid any uncontrollable chemical cues from fish, all areas upstream of the buffer reaches were kept fish-free by electric fishing.

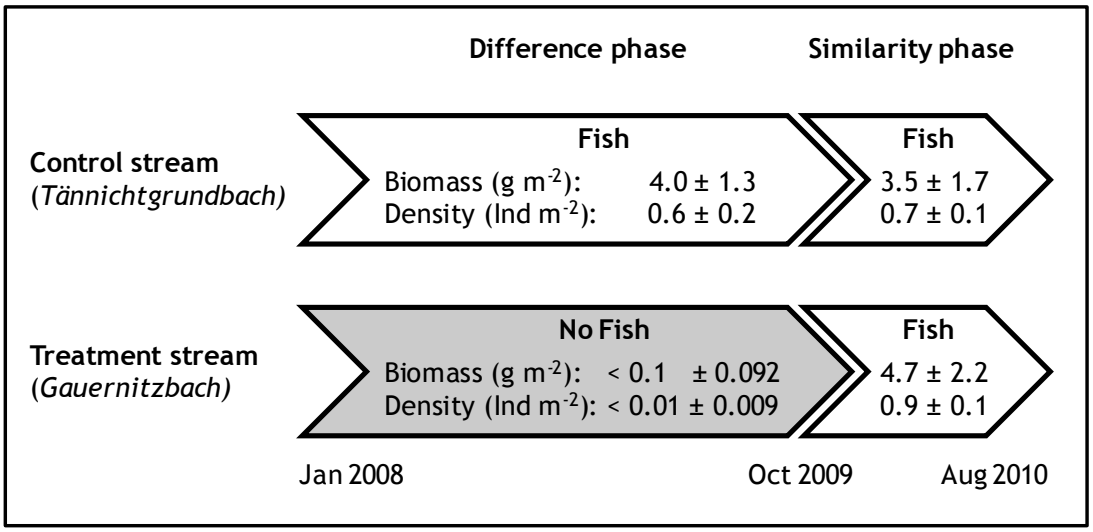

Figure 3.1 Experimental phases in the two streams and mean (± SD, n = 3) biomass and density in fresh weight of the benthivorous fish during this time.

Our study was conducted from January 2008 to August 2010. During this period the treatment stream was characterised by low fish biomass in the difference phase (January 2008 to October 2009) and high fish biomass in the similarity phase (November 2009 to August 2010) when fish stock was similar to the control stream due to restocking with benthivorous fish (Figure 3.1). In contrast to the classic BACI (before-after-control-impact) design, fish stocks were manipulated during both experimental phases to maintain the desired fish densities. Furthermore, the difference phase preceded the similarity phase because of the above mentioned ongoing long-term experiment on predation effects of benthivorous fish on benthic invertebrates (for details see Winkelmann et al., 2011).

Field sampling and laboratory analysis

The invertebrate communities were sampled every 28 days with a Surber sampler (0.12 m^2, 500 μm mesh size) from a randomly chosen pool/riffle sequence within the experimental reaches of each stream (3 samples in a pool and a riffle, respectively) and stored in 80 % ethanol. In the laboratory, benthic invertebrates were identified to the lowest practical taxonomic level. Individuals were enumerated and body length was measured to the nearest 0.1 mm with a stereomicroscope. For samples exceeding 100 individuals only 50 to 100 specimens were measured. Water quality was monitored at least every 28 days with WTW probes for electrical conductivity, oxygen concentration and pH (Weinheim, Germany, LF340, Oxi96 and pH196). Water temperature was measured at 15 min intervals using HOBO data loggers (Onset, Massachusetts, USA). For phosphate concentrations 100 mL stream water were filtered (cellulose acetate, 0.45 μm) and transported to the laboratory for further analysis.

Sampling for stable isotope analysis was conducted in May 2008 and May 2009 by picking five individuals of mayfly larvae *B. rhodani*, *R. semicolorata*, *Ecdyonurus* sp. and *E. ujhelyii* each from their respective substrata. All specimens were placed individually in 2 mL-Eppendorf-tubes, immediately frozen in liquid nitrogen, and stored at -80 °C until further processing. As possible food resources we collected three separate samples for each benthic algae, leaf litter and fine particulate organic material (FPOM, grain size < 1 mm). Anticipating different stable isotope values, filamentous (algal overstory) and closely attached (algal understory) algae were sampled separately from overgrown rocks. FPOM was collected by suction using

a big pipette from low current regions at the stream bottom. In the laboratory, potential food sources were cleaned under tap water and any attached animals were removed before samples were frozen. The closely attached algae which formed the algal understory were removed from stones with a brush using a small amount of tap water. Following 10 minutes of centrifugation (MLP K 26 D), an algae pellet was obtained and frozen. For each mayfly larvae, body length (to the nearest 0.1 mm) was measured and guts were removed. All samples (food resources and dissected mayfly larvae) were freeze-dried (Alpha 1-2, Martin Christ, Germany) at -55 °C for 24 h and ground into a homogenous powder. 3-5 mg of each resource sample and 0.5-1 mg of each mayfly sample were filled into small tin capsules (HEKAtech GmbH, 5x9 mm). Stable isotope analyses were performed at the Institute of Environmental Change and Society (IECS) of the University of Regina (Canada) using a Finnigan-Mat Delta Plus isotope ration mass spectrometer. All stable isotope values were expressed in the δ notation where $\delta^{13}C$ or $\delta^{15}N = ([R_{sample} / R_{standard}]-1) \times 1000$, and R is the ratio of $^{13}C / ^{12}C$ or $^{15}N / ^{14}N$. Isotopic composition is defined as parts per thousand (‰) and values are reported relative to the global $\delta^{13}C$ and $\delta^{15}N$ standards PeeDee *Belemnitella americana* (VPDB) and atmospheric nitrogen, respectively. Analytical accuracy for both stable isotopes was 0.2 ‰ or less.

Periphyton was sampled by randomly choosing three stones from the riffle immediately upstream of the invertebrate sampling. Periphyton biomass was sampled every 14 days during the whole study period. To determine chlorophyll-*a* concentration and periphyton composition, periphyton was removed from stones by carefully brushing with tap water. The obtained suspension was topped up to 65 mL. For chlorophyll-*a* analysis 1 or 2 mL of the suspension (depending on periphyton biomass) was filtered through glass fibre filters (Sartorius MGF) with suction pressure not exceeding 0.3 atm. Filters were stored at -20 °C (maximum 5 months). Extraction and fluorimetric analysis were done according to Wasmund et al. (2006) and Ritchie (2008). Briefly, filters were homogenised (Ultra Turrax, IKA, Staufen, Germany), extracted for at least 18 hours in buffered 96 % ethanol and measured spectrofluorometrically (LS 50B Luminescence spectrometer, Perkin Elmer, Massachusetts, USA).

The taxonomical analyses of periphyton were performed on pooled samples for each stream/sampling date combination. To estimate the quantitative composition of periphyton (volume proportions) the Lugol-fixated samples were examined

microscopically (in triplicate). To analyse diatoms in more detail, inorganic shells were cleaned from the organic proportion of the sample by boiling in concentrated sulphuric acid and small additions of potassium nitate. After washing, diatoms were mounted on slights using Naphrax (Biologie-Bedarf Thorns, Deggendorf, Germany). This procedure was conducted in collaboration with Uta Raeder (Technical University of Munich) based on her own work (Raeder and Busse, 2001) according to the diatom-preparation method of Van der Werff (1955). Diatoms were identified to the lowest practical taxonomical level (species or genus) with a microscope of 1000 x magnification (Olympus BX40, Hamburg, Germany) and counted using as many transects as were necessary to examine at least 350 cells. No distinction was made between live and dead diatoms before acid cleaning.

For mayfly gut content analysis 5 larvae (without black wing pads) of each species were sampled in both streams every 14 days throughout the duration of a complete mayfly generation (September 2008 to July 2009, when the control and treatment streams differed in fish density; difference phase, Figure 3.1). Larvae were placed individually in 2 mL Eppendorf tubes, frozen immediately in liquid nitrogen and stored at -80 °C. After thawing, midgut and foregut of each mayfly were carefully removed under a dissecting microscope (TSO-Gerätebau, Germany). The gut contents were squeezed into some drops of tap water and the resulting solution was transferred into an Eppendorf tube filled with tap water (final volume: 1 mL), gently homogenised and thereafter transferred into a sedimentation/counting chamber (diameter: 25 mm, volume: 1 mL). After sedimentation, algal cells were identified and counted using the Utermöhl method (Utermöhl, 1958) under an inverted microscope (Wilovert, TSO, Germany) by counting two diameter transects at 400 x magnification (Tümpling and Friedrich, 1999). For detailed examination of algae a higher magnification was used. The counting units were usually cells and in the case of filamentous species only fragments > 100 µm were counted. Between the different growth forms of *Audouinella* sp. (filamentous and chantransia forms) were not distinguished.

To evaluate the spatial structure of periphyton assemblages, algal cells were categorised into four groups based on vertical locations in the algal community (relative height). Group *At* included attached and motile algae species located in the lowest algal layer (algal understory). Group *St* formed the middle layer and included stalk and tube forming species. Chain and rosette forming species (group *Ch*) as

well as filamentous species (group *Fi*) constituted the uppermost layer also called algal overstory (Table 3.3).

Table 3.3 Taxonomic composition of periphyton algae obtained from the gut contents of studied mayflies distinguished into four categories based on their growth habits and vertical locations in the periphyton mat. Groups *At* and *St* were various small and intermediate diatoms located in the lowest and middle layer of the periphyton mat (algal understory). Group *Ch* and *Fi* were large filamentous algae which appeared in the uppermost layer of algal community as algal overstory (inspired by Steinman, 1996 and Katano et al., 2002).

Algal physiognomic group	Class and taxon
Attached/motile (*At*)	Diatoms: *Achnanthes* sp., *Amphora* sp., *Brachisyra* sp., *Cocconeis placentula*, *Navicula* sp., *Nitzschia* sp., *Surirella brebissonii*
Stalk/tube forming (*St*)	Diatoms: *Cymbella* sp., *Gomphonema* sp., *Rhoicosphenia abbreviata*
Chain/rosette forming (*Ch*)	Diatoms: *Fragillaria brevistriata*, *Meridion circulare*
Filamentous (*Fi*)	Rhodophyta: *Audouinella* sp. Cyanophyta (Cyanobacteria): *Phormidium* sp.

Data analysis

Individual biomass of invertebrates was calculated as dry mass by using length-weight relationships (Meyer, 1989; Burgherr and Meyer, 1997; Benke et al., 1999). Total grazer biomass was derived by adding the biomass of each species corrected by the factor of food preference as listed in Schmedtje and Colling (1996) and Tachet et al. (2000). For example, a grazer species feeding 50 % on periphyton was multiplied with the factor of 0.5. Hence, 'grazer biomass' represents the functional biomass of grazers (Table 3.2).

The influence of benthivorous fish on composition of periphyton algae and diatoms was assessed using randomised intervention analysis (RIA) (Carpenter et al., 1989). RIA uses paired, before-and-after time-series data from a manipulated and a control system to analyse alterations caused by the manipulation (for manipulation

scenarios see Figure 3.1). Specifically, for each sampling occasion between-stream differences were calculated for periphyton biomass, relative abundance of total diatoms and diatoms of the group *St* (stalk/tube forming diatoms; specific diatoms). The same procedure was used for between-stream differences of total grazer biomass (as functional biomass of grazers) and the proportions of *B. rhodani* and *Ecdyonurus* sp. (preferring stalk/tube forming diatoms) to total grazer biomass. The results were compared between the difference and similarity phases (randomisation test, R Development Core Team, Version 3.0.3, 2014) to analyse whether there was a non-random change in the mean between-stream differences (for details of experimental phases see Figure 3.1).

The differences in gut content composition among mayfly species (factor 1) and between manipulated streams (factor 2) were tested via multivariate permutation test based on a test statistic analogous to Fisher´s *F*-ratio (Anderson, 2001; function 'adonis' integrated into the software R, 1000 permutations). The permutation test was stratified over sample time, which permitted the comparison only within the sampling dates and not across all dates. The gut content data (counts of algae cells per mm of larval body length) were standardised ($\sqrt[4]{}$) and the multivariate data set was converted into a similarity matrix based on the Bray Curtis Euclidean distance measure (see requirements in Anderson, 2001). We used a multiple comparison test (Tukey-HSD test with Bonferroni correction) to compare > 2 treatments within a significant factor (e.g., mayfly species‘). Finally, a similarity percentage analysis (“SIMPER”, Primer 6) was followed to identify those algal groups in the gut contents contributed most to the differences between treatments.

To enable the comparison of gut contents of individual mayflies we applied a conversion factor for the raw counts based on Utermoehl chamber volume. To enhance comparability between individuals of different sizes, algal abundances were reported per mm of body length. Subsequently, relative proportions were calculated for each algal taxon to assess the diet composition of mayfly larvae.

Selection of different food types by mayflies (*r*; such as groups of epilithic algae, physiognomic groups of diatoms and diatom species) in relation to their abundance or availability in the environment (*p*) was estimated using the electivity index (*E**) of Vanderploeg and Scavia (1979a; 1979b) (Equation 3.1). The indices of electivity or feeding preference based on the proportions of food *i* in the diet (r_i) and in the environment (p_i, Equation 3.1 and 3.2).

$$E^* = [W_i - (1 / n)] / [W_i + (1 / n)] \quad \textbf{(3.1)}$$

based on the number of available food types and on the selectivity coefficient W_i as:

$$W_i = [r_i / p_i] / [\sum_i r_i / p_i] \quad \textbf{(3.2)}$$

To counteract the alpha error accumulation and to adapt the multiple alpha levels, the selectivity indices were tested against zero using Wilcoxon tests with Bonferroni-Holm correction. Take into account the different algal counting methods for stream and gut algae, only relative algal abundances were used to calculate electivity indices.

Differences in $\delta^{15}N$ and $\delta^{13}C$ values between streams and among grazers were tested with a two-way ANOVA followed by a multiple comparison test (Tukey-HSD test with Bonferroni correction).

All statistical tests were performed using R (R Development Core Team, Version 3.0.3, 2014) and SigmaPlot (Systat Software Inc., Version 11.0) was used to prepare the graphs.

3.4 Results

Trophic cascade

In general, our field experiment indicated a classical trophic cascade induced by benthivorous fish species stone loach and gudgeon. Due to the reduction of total grazer biomass, periphyton was released from grazing pressure and consequently, developed a higher biomass (Figure 3.2). However, via changes in community composition of grazers, namely the facilitation of *Baetis rhodani* and *Ecdyonurus* sp., benthivorous fish induced a change in periphyton community, reducing the proportion of stalked or tube forming diatoms (Figure 3.2). The specific results leading to this general view are described below.

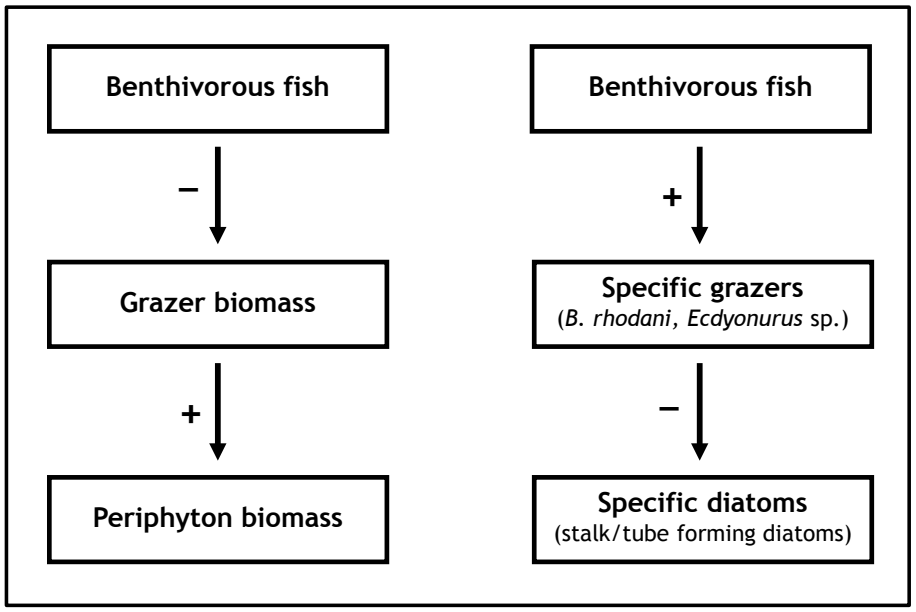

Figure 3.2 Summary of positive/negative cascading effects during the fish scenario. On the left side are shown the effects on trophic levels in general: from benthivorous fish via the grazer guild to periphyton. On the right side are displayed the effects on community level: from benthivorous fish via specific grazer species to specific physiognomic algal group of stalk/tube forming diatoms. Reduction of the lower trophic level is indicated by '-' and increase by '+'.

Fish effects on grazer biomass and specific grazer proportion

The presence of benthivorous fish in the treatment stream (similarity phase) reduced the total grazer biomass significantly. This was indicated by the significant difference of the between-stream difference (treatment stream minus control stream) of total grazer biomass between the experimental phases (Figure 3.3a, $p = 0.046$, randomisation test). In contrary to our initial expectation, the difference was larger in the similarity phase (fish in both streams). This might result from the fact that there was a relatively high natural difference between the streams with relatively lower grazer biomass in the treatment stream in the similarity phase, when fish stock was similar. In the difference phase (no fish in treatment stream), grazer biomass was higher in the treatment stream relative to the control stream resulting in a reduced between-stream difference (Figure 3.3a).

In addition, there are considerable inter-annual variations in benthic grazer biomass (Table 3.2). Assuming that these variations are the same in the two streams, grazer biomass in the treatment stream is assessed relative to the control stream and plotted as between-stream difference (Figure 3.3a).

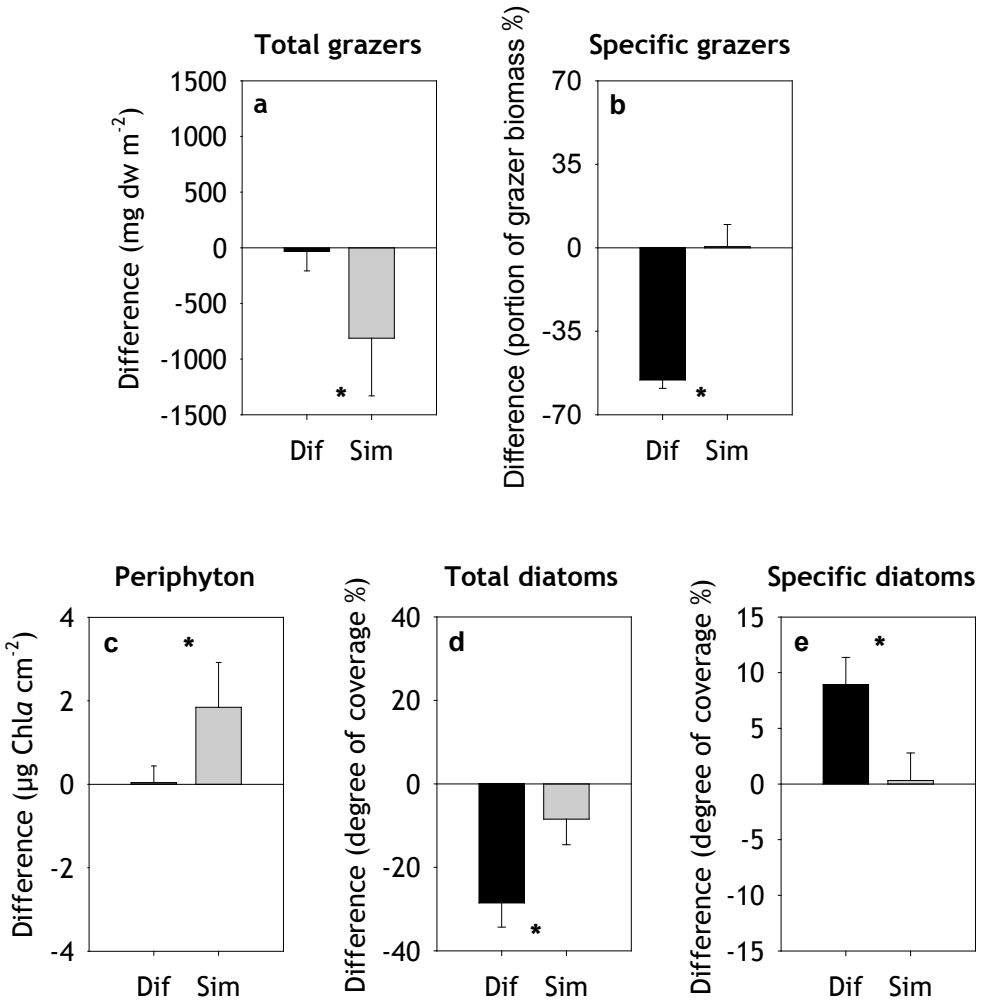

Figure 3.3 Between-stream differences (means ± 1 SE over the study period) of **(a)** total grazer biomass (n = 23/9), **(b)** proportion of biomass of these grazer species which prefer stalk/tube forming diatoms (*B. rhodani* and *Ecdyonurus* sp. n = 23/7), **(c)** periphyton biomass (n = 38/11), **(d)** proportion of total diatom coverage (n = 35/11), and **(e)** proportion of coverage of stalk/tube forming diatoms (n = 34/7). The between-stream difference is calculated as treatment stream minus control streams during the difference (Dif) and similarity (Sim) phases, for example: $Biomass_{\text{treatment stream}} - Biomass_{\text{control stream}}$. Significant differences are indicated by *.

Following the same principle as described above, the presence of benthivorous fish significantly affected the proportion of those grazers, preferring stalk/tube forming diatoms (*B. rhodani*, *Ecdyonurus* sp.). This grazer fraction significantly increased in the presence of fish relative to the total grazer biomass (Figure 3.3b) which was indicated by a significant higher between-stream difference of biomass portion of those grazers in the difference phase (no fish in treatment stream) than in the similarity phase (Figure 3.3b, $p = 0.0003$, randomisation test).

Fish effects on periphyton biomass and composition

The presence of benthivorous fish in the treatment stream (similarity phase) increased the biomass of periphyton and the biomass of total diatoms. This was indicated by the significantly higher between-stream difference of periphyton biomass and the significantly lower between-stream difference of total diatom biomass in the similarity phase than in the difference phase, when no fish were present in the treatment stream (Figure 3.3c and Figure 3.3d, $p = 0.038$, $p = 0.031$, randomisation test). The change in the mean between-stream difference in diatom biomass was not caused by a change in diatom dominance in the treatment stream between the phases as might be expected (similarity phase: 32.1 ± 8.1 %, $n = 11$; difference phase: 29.8 ± 4.7 %, $n = 35$, mean ± SE,). Instead, the diatom fraction seemed lower in the control stream during the similarity phase (40.5 ± 9.8 %, $n = 11$) than during the difference phase (58.3 ± 4.7 %, $n = 35$) although the fish stocks remained unchanged. This might be caused by inter-annual variability and is corrected for by analysing only the between-stream differences.

In contrast to the increased diatom biomass, the fraction of the stalk/tube forming diatoms was reduced by benthivorous fish. This was indicated by the significantly lower between-stream difference in coverage of these specific diatoms in the similarity phase than in the difference phase, when no fish were present in the treatment stream (Figure 3.3e, $p = 0.049$, randomisation test). During the similarity phase (fish in both streams) the degree of coverage of this diatom group was very similar in the control (46.4 ± 8.8 %, mean ± SE, $n = 7$) and treatment (46.7 ± 8.5 %, $n = 7$) streams resulting in a low mean between-stream difference.

During the difference phase, however, the between-stream difference was much higher (Figure 3.3e), indicating that the proportion of stalk/tube forming diatoms was higher in the treatment stream during the absence of fish than in the control stream (treatment: 24.6 ± 3.1 %, n = 34, control: 15.7 ± 4 %, n = 34, mean ± SE).

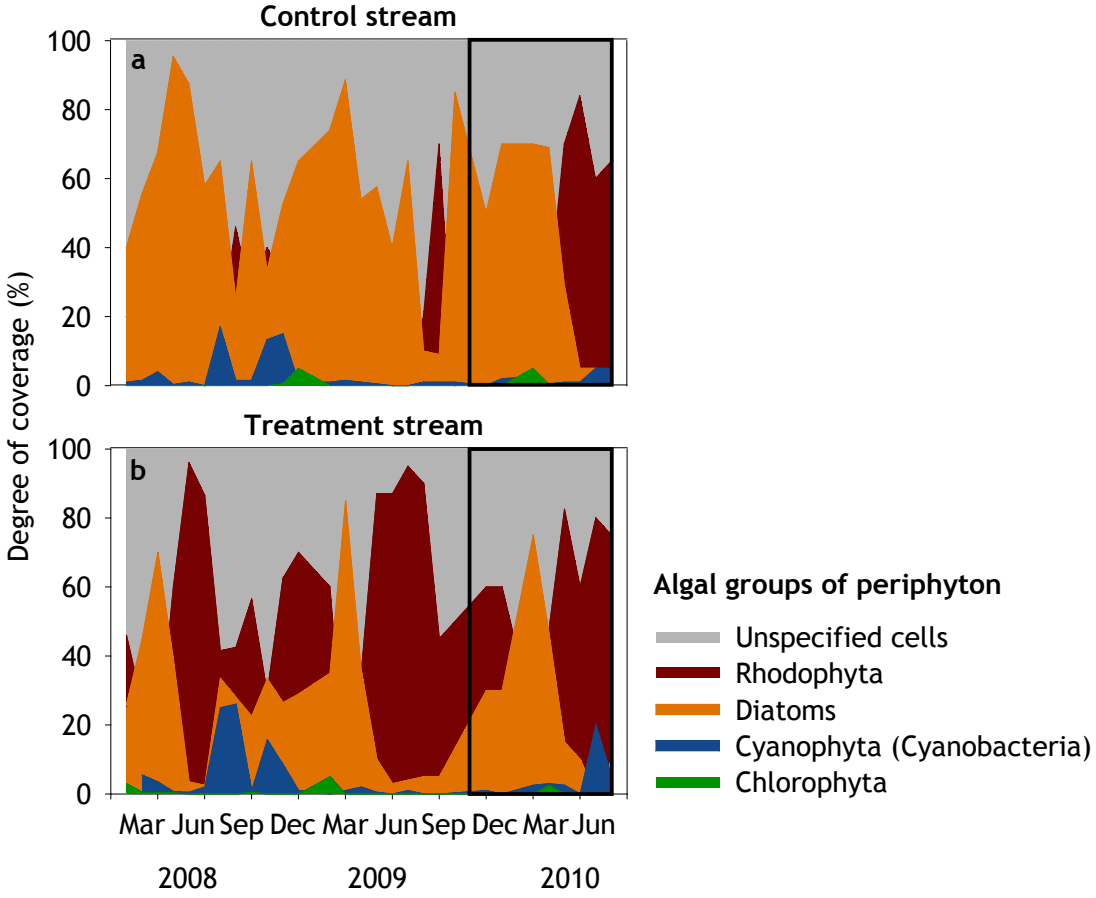

Figure 3.4 Composition of periphyton algae in the **(a)** control, and **(b)** treatment streams, during the difference (n = 35) and similarity (n = 11, characterised by black frames) phases.

The natural community composition of periphyton in both streams consisted mainly of diatoms and rhodophytes (Figure 3.4). During the whole field study period (January 2008 to August 2010) a dominance of diatoms was observed in the control stream with continuously high fish densities, where diatoms accounted for 51 ± 4 % (mean ± SE, n = 46) of total algal coverage (Figure 3.4a).

In contrast, in the treatment stream rhodophytes were most abundant with 57 ± 5 % (mean ± SE, n = 35, difference phase) of total algal coverage and during the similarity phase with 61 ± 6 % (mean ± SE, n = 11) of total algal coverage (Figure 3.4b).

Seasonal dynamics of growth forms and taxonomic composition of diatoms were generally similar between the streams and not affected by fish predation (Figure 3.5 and Figure 3.6).

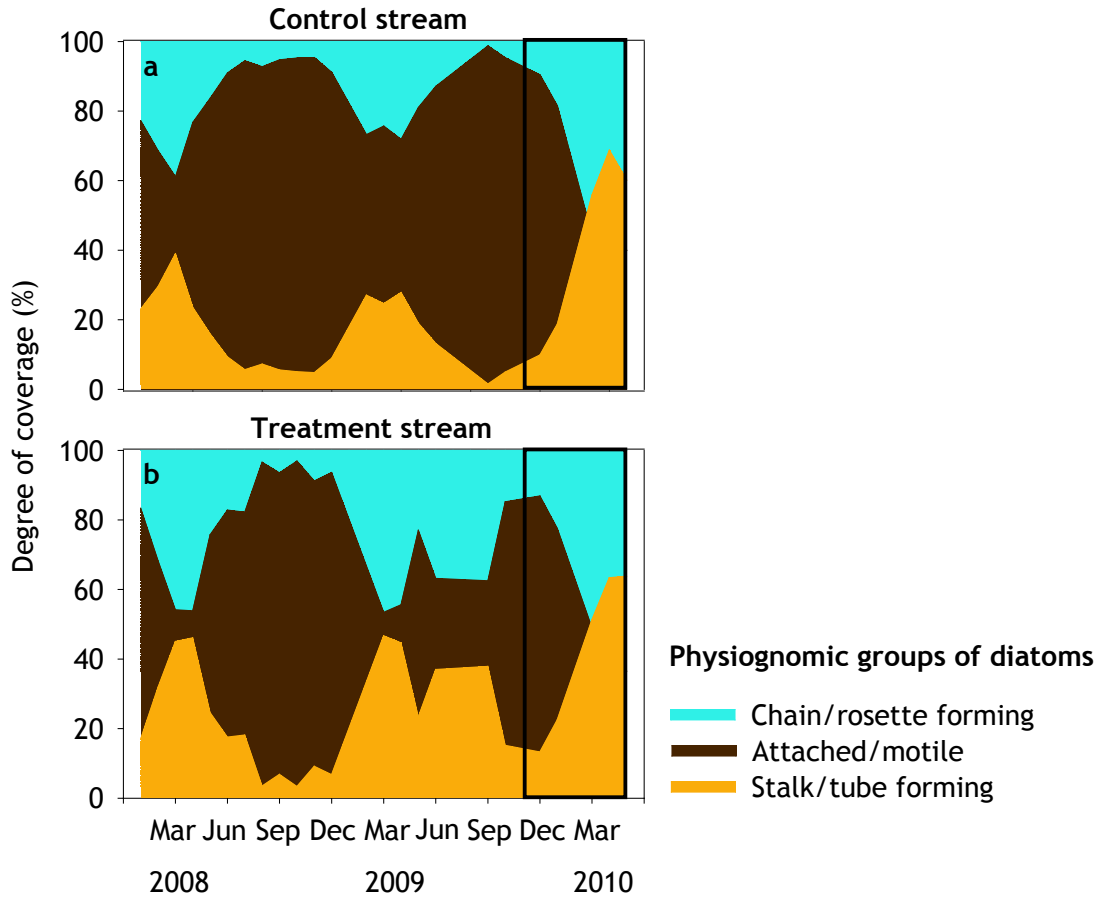

Figure 3.5 Diatom physiognomic groups in the (a) control, and (b) treatment streams during the difference (n = 34) and similarity (n = 7, characterised by black frames) phases.

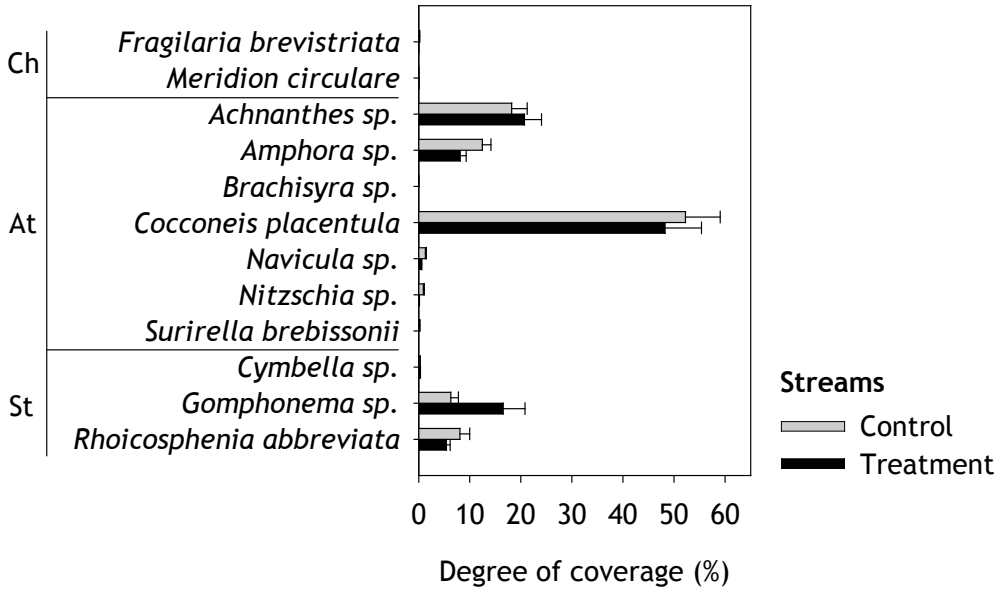

Figure 3.6 Diatom composition (mean ± 1 SE, n = 17 per stream) within the periphyton algae. Chain/rosette forming (Ch), attached/motile (At), and stalk/tube forming (St) growth forms obtained in the control (grey bars), and treatment (black bars) streams during a complete mayfly generation (September 2008 to July 2009).

Grazer diet composition and fish effects on dietary

Generally, our focal grazer species *B. rhodani*, *R. semicolorata*, *Ecdyonurus* sp., *E. ujhelyii* and seemed to feed mainly on periphyton, as was suggested by stable isotope and gut content analyses. For both streams the similarity of $\delta^{13}C$ values between resources and animals suggested that Heptageniidae (*R. semicolorata*, *Ecdyonurus* sp., *E. ujhelyii*) fed on periphyton, especially the closely attached parts of the periphyton mat (algal understory; Figure 3.7). Species of *B. rhodani* (Baetidae) potentially used filamentous parts (algal overstory) of periphyton besides other periphyton parts as the higher similarity between the carbon signatures of mayfly species and filamentous algae suggested (Figure 3.7). Based on $\delta^{15}N$, the use of mainly autochthonous resources by the four mayfly grazers in both streams is likely because an average $\delta^{15}N$-increase of 2.4 ‰ was observed between studied species and the algae parts of periphyton (Figure 3.7).

Among periphyton, diatoms were the dominant grazer diet as gut content analysis revealed that diatoms represented 97.4 ± 3.7 % of total gut content

(mean ± SD, n = 660) with no significant differences between the streams (Figure 3.8). Further, the studied grazer species significantly preferred diatoms and significantly avoided filamentous rhodophytes and cyanophytes (Figure 3.9; see Table 3.4: $p < 0.05$, Wilcoxon test).

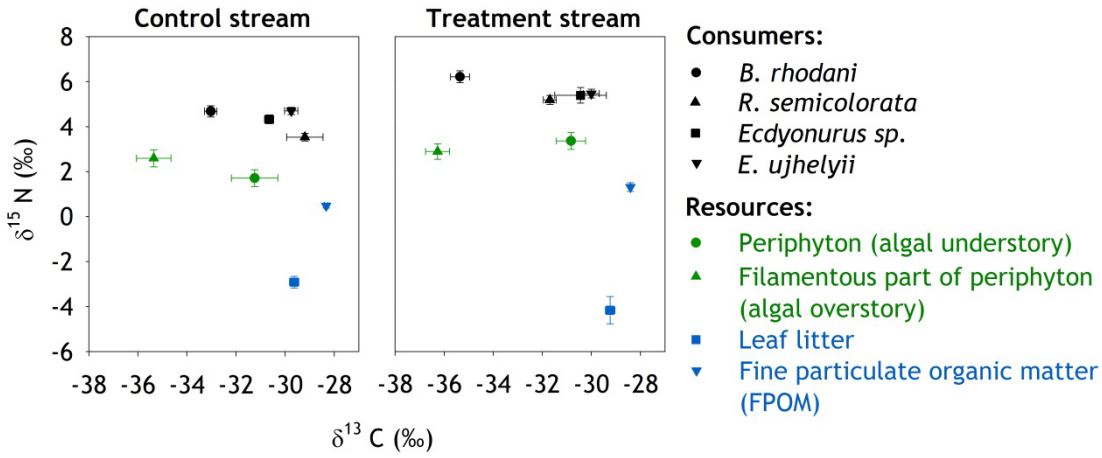

Figure 3.7 Stable isotope characterisation (mean ± 1 SE, spring 2008 and spring 2009) of important carbon sources (n = 3 per stream) and studied mayfly larvae (n = 5 per stream) as primary consumers within the food web of the control and treatment streams.

In respect to diatoms, *B. rhodani* and *Ecdyonurus* sp. significantly preferred stalk/tube forming growth forms, whereas *E. ujhelyii* significantly preferred attached/motile diatoms (Figure 3.9; see Table 3.4: $p < 0.05$, Wilcoxon test). Chain/rosette forming diatoms were mostly avoided by the studied species (Figure 3.9; see Table 3.4: $p < 0.05$, Wilcoxon test). Overall, diatom taxa *Cocconeis placentula* and *Achnanthes* sp. were the most abundant food items in the gut contents of all studied mayfly larvae (*B. rhodani*: 63 %, *R. semicolorata*: 68 %, *E. ujhelyii*: 76 %, *Ecdyonurus* sp.: 74 % of the average gut content, n = 190 per mayfly species, *R. semicolorata*: n = 110). The stalk/tube forming diatoms *Gomphonema* sp. and *Rhoicosphenia abbreviata* were also frequently ingested (*B. rhodani*: 32 %, *R. semicolorata*: 24 %, *E. ujhelyii*: 18 %, *Ecdyonurus* sp.: 22 % of the average gut content, n = 190 per mayfly species, *R. semicolorata*: n = 110). No individual diatom species was significantly preferred by the studied grazers, with the caveat that *C. placentula* was positively selected by *Ecdyonurus* sp. in the control stream (Figure 3.10; see Table 3.4: $p < 0.05$, Wilcoxon test). In contrast, many diatom species were negatively selected by mayflies in both streams,

especially species with low abundances such as *Brachisyra* sp., *Cymbella* sp. and *Meridion circulare* (Figure 3.10; see Table 3.4: $p < 0.05$, Wilcoxon test).

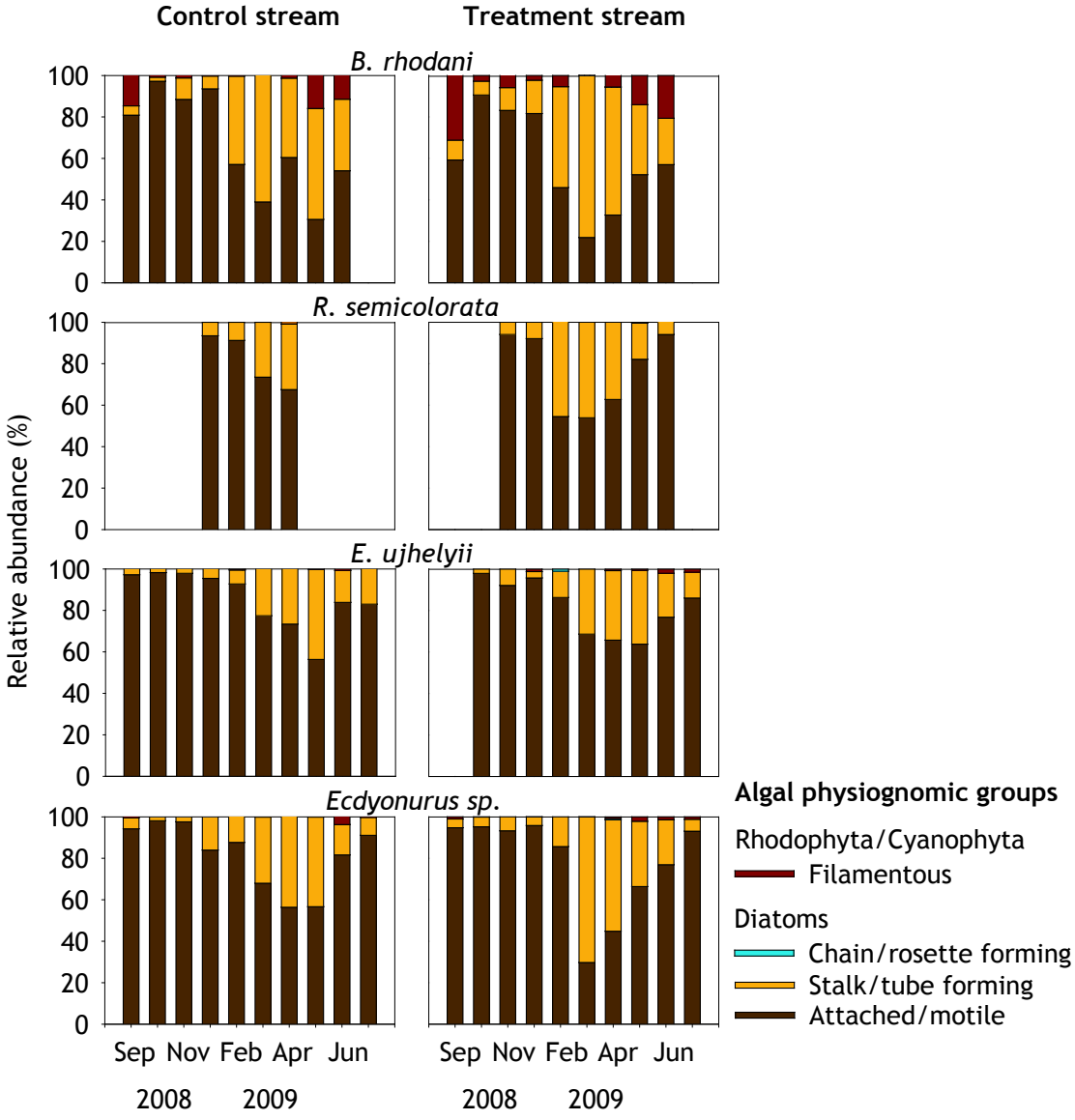

Figure 3.8 Algal physiognomic group proportions as relative abundance in mayfly gut contents considering the control and treatment streams during a complete mayfly generation (September 2008 to July 2009, n = 10 per month and stream). Certain months are without data because no larvae were found at that time.

Table 3.4 Summary of adjusted *p*-values as a result of the Wilcoxon tests with Bonferroni-Holm correction of the Vanderploeg and Scavia's electivity indices during a complete mayfly generation in the control (Con) and treatment (Tre) streams (n = 18 per stream, *R. semicolorata*: n = 8 in the control stream). Italic values indicate significant avoidance or preference, NA = not addressed.

	B. rhodani		*R. semicolorata*		*E. ujhelyii*		*Ecdyonurus sp.*	
	Con	Tre	Con	Tre	Con	Tre	Con	Tre
Algal groups								
Diatoms	*0.01*	*0.01*	NA	*0.04*	*0.01*	*0.02*	*0.01*	*0.01*
Rhodophyta	*0.01*	*0.01*	NA	*0.04*	*0.01*	*0.02*	*0.01*	*0.01*
Cyanophyta (Cyanobacteria)	*0.01*	*0.01*	NA	*0.04*	*0.01*	*0.01*	*0.01*	*0.01*
Diatom groups								
Chain/rosette forming	*0.01*	*0.02*	0.56	0.09	*0.01*	*0.047*	*0.01*	*0.047*
Attached/motile	0.73	0.55	0.56	0.09	*0.02*	*0.047*	0.07	0.38
Stalk/tube forming	*0.01*	*0.02*	1.0	0.56	0.50	0.58	*0.04*	0.22
Diatom species								
F. brevistriata	*0.04*	0.07	1.0	0.24	0.06	0.13	*0.04*	0.07
M. circulare	*0.04*	0.08	0.86	0.26	*0.04*	0.14	*0.04*	0.12
Achnanthes sp.	0.49	1.0	1.0	1.0	0.20	1.0	0.11	1.0
Amphora sp.	0.12	0.12	1.0	0.26	0.06	0.14	*0.04*	0.10
Brachisyra sp.	*0.04*	0.07	0.86	0.24	*0.04*	0.13	*0.04*	0.07
C. placentula	1.0	0.75	1.0	0.26	0.06	0.23	*0.04*	0.10
Navicula sp.	0.66	0.88	1.0	1.0	1.0	1.0	0.22	1.0
Nitzschia sp.	0.06	0.08	0.88	0.26	0.93	0.17	0.10	0.08
S. brebissonii	0.06	0.08	1.0	0.26	0.08	0.17	0.10	0.08
Cymbella sp.	*0.04*	0.07	0.86	0.26	*0.04*	0.13	*0.04*	0.07
Gomphonema sp.	1.0	0.59	1.0	1.0	0.93	1.0	0.41	1.0
R. abbreviata	1.0	0.12	1.0	1.0	1.0	1.0	0.41	0.78

Gut content composition significantly differed among grazer species and between streams as was indicated by the multivariate permutation test (factor 'species': df = 3, F = 174.3, p < 0.001; factor 'stream': df = 1, F = 25.63, p = 0.03; stratified by dates Figure 3.8). The gut content of *B. rhodani* differed significantly from the other grazer species (*Ecdyonurus* sp.: p = 0.045, *R. semicolorata*: p = 0.005; Tukey-HSD test) due to the larger proportion of stalk/tube forming diatoms and filamentous algae in the gut. This was supported by a SIMPER analysis that showed a high contribution of stalk/tube forming diatoms (40 - 45 %) and of filamentous algae (21 - 22 %) to these differences. No general between-stream difference in gut content was found for individual species without considering time (Tukey-HSD test, p > 0.05).

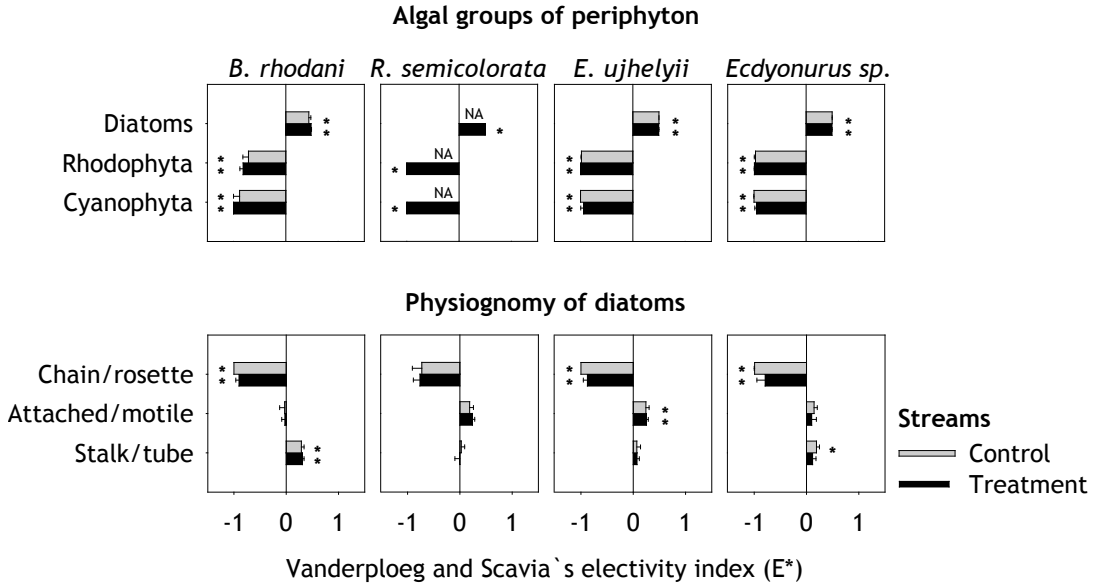

Figure 3.9 Mean electivities (± 1 SE) for periphyton algae during a complete mayfly generation (September 2008 to July 2009) in the control (grey bars) and treatment (black bars) streams calculated with the Vanderploeg and Scavia's electivity index. Range between plus one is indicating preference, minus one is indicating avoidance, and zero for random feeding. Significant preference or avoidance is indicated by * with a p-value < 0.05, NA = not addressed (Wilcoxon test with Bonferroni-Holm correction, n = 18 per stream, *R. semicolorata*: n = 8 in the control stream).

However, in the spring months March, April and May (year 2009) the gut content of *B. rhodani* and *Ecdyonurus* sp. showed a higher proportion of stalk/tube forming algae in the treatment stream than in the control stream (Figure 3.8). This parallels the

slightly higher proportion of this algal group on total diatom abundance in the treatment stream during these months (Figure 3.5), and is also supported by the overall average contribution of this group of nearly 46 % to the between-stream difference (SIMPER analysis).

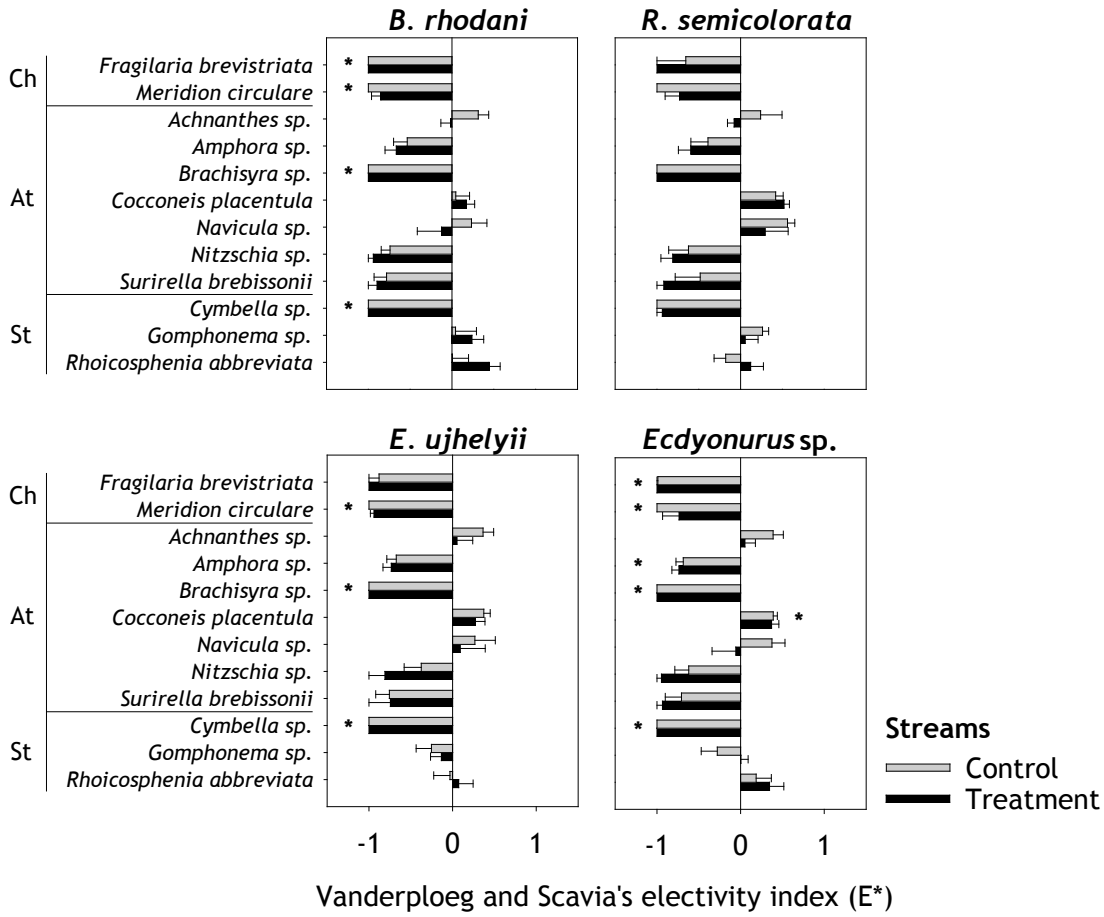

Figure 3.10 Mean electivities (± 1 SE) for identified diatom species (Ch: chain/rosette forming, At: attached/motile, St: stalk/tube forming) during a complete mayfly generation (September 2008 to July 2009) in the control (grey bars) and treatment (black bars) streams calculated with the Vanderploeg and Scavia's electivity index. Range between plus one is indicating preference, minus one is indicating avoidance, and zero for random feeding. Significant preference or avoidance is indicated by * with a p-value < 0.05 (Wilcoxon test with Bonferroni-Holm correction, n = 18 per stream, *R. semicolorata*: n = 8 in the control stream).

There was no clear difference of dietary preferences between the control and treatment streams as the patterns of positive and negative selection of periphyton algal

groups, physiognomic diatom groups as well as diatom species did not differ between the streams (Figure 3.8 and Figure 3.9). However, gut content analysis was limited to the period from September 2008 to July 2009, and therefore data exist only for the difference phase (fish present only in the control stream; Figure 3.1).

Environmental conditions

Environmental factors, which might have affected algal growth or grazer biomass did not show any change in between-stream differences between the similarity phase and the difference phase, indicating that these conditions were most probably not responsible for the observed changes between the phases (Table 3.5). There was, however, considerable inter-annual variability. Light supply, which can be assumed to have a direct effect on periphyton growth rate and community composition, differed considerably between the difference and similarity phases especially in spring. In May 2010 (similarity phase) the lowest sunshine duration since 1917 was measured in the experimental region with only 99.5 hours sunshine. By comparison, the average duration of sunshine for May was measured with 216.8 ± 4.9 h (monthly mean ± SE, years 1917 to 2014). In May 2008 (difference phase) 263.7 hours and in May 2009 (difference phase) 225 hours sunshine were recorded at the weather station Dresden-Klotzsche.

Table 3.5 Mean values (± SE, *n* = difference/similarity phase) of environmental conditions phosphate (*n* = 23/8), water temperature (*n* = 36/13), pH (*n* = 37/12) and discharge (*n* = 38/9) during the difference and similarity phases in the control and treatment streams. Significance levels (*p*) indicate the between-stream differences between the difference and similarity phases (randomisation test).

	Difference phase		**Similarity phase**		***p***
	Control	Treatment	Control	Treatment	
Phosphate (µg P L^{-1})	50.7 ± 4.7	36 ± 4.5	48.1 ± 10.4	29.2 ± 6.8	0.83
Temperature (°C)	9.7 ± 0.7	9.3 ± 0.8	10.7 ± 1.5	10.3 ± 1.6	0.44
pH	7.9 ± 0.1	8.2 ± 0.03	8 ± 0.1	8.4 ± 0.03	0.51
Discharge (L s^{-1})	64 ± 9.7	38 ± 6	73 ± 11.5	46 ± 7.2	0.62

3.5 Discussion

Direct fish effects

As predicted in our first hypothesis (**Hypothesis 1a**), benthivorous fish reduced the benthic grazer biomass and thus indirectly increased the periphyton biomass. The occurrence of a trophic cascade in this ecosystem had been confirmed in an earlier study focusing on seasonal aspects (Winkelmann et al., 2014), while the present study investigated the strength of direct and indirect trophic effects to quantify the impacts of benthivorous fish (presence/absence). A more detailed analysis of grazer biomass changes revealed that some grazer species benefited from the presence of fish. Although total grazer biomass was reduced in fish presence, the proportion of the two of the studied mayfly species (*Baetis rhodani* and *Ecdyonurus* sp.) increased relatively to total grazer biomass. There are at least two potential reasons for this observation. First, benthivorous fish species gudgeon and stone loach are known to feed selectively and prefer certain prey species, especially selecting small and highly abundant species (Worischka et al., 2015). This would explain at least the facilitation of *Ecdyonurus* sp. because this taxon belongs to medium- and large-size classes of prey and was not particularly abundant in the ecosystems. Second, grazer species express different predator avoidance strategies. Larvae of *B. rhodani* for example, are able to change their feeding periodicity pattern in order to avoid benthic feeding fish or to increase their drift activity at night as post-contact antipredator mechanism (e.g. Huhta et al., 2000; Miyasaka and Nakano, 2001; Schneider et al., 2014). Consequently, it is likely that this mayfly species was able to reduce the risk of predation.

Indirect density-mediated fish effects

Supporting our second hypothesis (**Hypothesis 2a**), presence of benthivorous fish induced significant changes in periphyton community composition most probably via their density-effects on benthic grazers. While total grazing pressure seems to have been reduced, the specific preference of the mayfly larvae *B. rhodani* and *Ecdyonurus* sp. resulted in a change of periphyton community due to their density increase relative to other grazer taxa. Overall, total diatoms benefited from fish presence, while the fraction of stalk/tube forming diatoms was reduced when benthivorous fish were present. The presence of benthivorous fish reduced the total grazer

biomass. Therefore it can be assumed that fish predation also reduced the mayfly biomass because mayflies represented the largest proportion of the grazers in both streams and generally, they showed a strong preference for diatoms as food items. Consequently, the observed increase in total diatom proportion during fish presence is interpreted to be a result of a generally reduced mayfly grazing pressure. However, although total grazer biomass was reduced in the presence of fish, we observed an increase in the proportion of two mayfly species (*B. rhodani*, *Ecdyonurus* sp.) preferring stalk/tube forming growth forms. Consequently, the grazing pressure on this diatom group also increased relative to other growth forms and the proportion of stalk/tube forming diatoms was heavily reduced in the treatment stream during fish presence. In the absence of fish however, we observed a dominance of rhodophytes which might be explained by competitive release due to the low diatom-density, making space, light and nutrients available (Liess and Hillebrand, 2004). The low diatom biomass is assumed to result from high grazer biomasses due to the absence of feeding pressure on the grazer species.

Contrary to our initial expectations, however, the observed differences in periphyton community composition between the experimental phases were at least partly caused by a change in the control stream, where fish stock was similar during the whole experiment. Therefore one could argue that the observed change in periphyton community composition was not associated with the change in fish stock, but rather by changes in environmental factors. Nevertheless, we are confident that the fish stock was responsible for the observed effects because large differences in environmental condition were accounted for by the experimental design. Any environmental factor that would selectively suppress diatom growth would have affected both streams similarly. In fact, we assume that the extremely low light supply in May 2010 resulted in an early dominance of rhodophytes in the control stream and prevented an increase in diatom density due to lower grazing pressure in the treatment stream. In May 2010 the lowest sunshine duration since 1917 was recorded in the experimental region; less than half the normal value.

Under normal circumstances, one would have expected a higher percentage of diatoms in the treatment stream due to the fish reducing grazer biomass and facilitating diatoms. This would have reduced the difference between the two streams in the similarity phase. However, the expected diatom dominance in the treatment stream was most likely prevented by the extreme weather situation, facilitating

rhodophytes which are generally better adapted to low-light conditions than diatoms (e.g. Bogorad, 1962; Brody and Brody, 1962; Fott, 1971). Despite this weather situation, there was still a lower difference between the two streams in the similarity phase because in both streams fish stock and the resulting grazing pressure were similar and diatom density was controlled mainly by light availability. Other environmental factors which could have affected only one stream such as phosphate concentration, pH or water temperature did not show any changes relative to between-stream differences.

Indirect trait-mediated fish effects

Changes in food choice, as possible trait changes due to fish presence were not observed in the present study. Selectivity patterns of ambient mayfly larvae did not differ between streams with or without benthivorous fish which contradicted our third hypothesis (**Hypothesis 2b**). However, trophic cascades in stream ecosystems, specifically quantitative changes of primary producers in response to predators (Dahl, 1998b; Forrester et al., 1999; Biggs, 2000; Winkelmann et al., 2014) can generally result from trait-mediated trophic effects (Peacor and Werner, 2001) when predator-induced behavioural shifts modify feeding activity. Especially reductions in food intake of mayflies as a consequence of predator avoidance behaviour in response to different predator types have been documented before (Culp et al., 1991; McIntosh, 1995; Schneider et al., 2014). The same principle might be applied to qualitative changes in periphyton assemblages due to predator presence. Potentially, behavioural changes of grazers resulting in different food preferences might have induced differences in periphyton community structure. However, although gut content analyses showed strong differences between the mayfly species, the effect of stream was weak indicating the lack of a conspicuous fish effect on grazer feeding preferences.

General feeding preferences

In our experimental systems, studied mayfly species avoided the algal overstory consisting of chain/rosette forming diatoms and filamentous algae (e.g. *Audouinella* sp., Rhodophyta). Dietary analyses revealed that these mayfly larvae significantly preferred diatoms of the thin (attached/motile diatoms) and middle (stalk/tube forming diatoms) periphyton layers. This observation is interpreted as a selection

of high quality food. Diatoms are generally considered a good quality food resource because they have a higher nutritional value than cyanophytes or even chlorophytes (Gregory, 1983; Hart, 1985; Feminella and Resh, 1991). Especially the high content of polyunsaturated lipids in diatoms seems to be crucial for the nutriation (Steinman et al., 1987b) because they are essential for benthic macroinvertebrates (Allan and Castillo, 2007b). This line of thought is supported by previous studies which found selective feeding including avoidance of cyanophytes by gastropods in enclosures and streamside flow-through channels (Nicotri, 1977; Rosemond, 1993; Rosemond et al., 2000) or the preference of *Nitzschia* spp., *Surirella spiralis* and *Navicula cryptocephala* by the grazing mayfly *Ameletus validus* (Hill and Knight, 1987). However, there are other observations indicating intensive grazing on freshwater rhodophytes, especially for the species *Audouinella violacea* (Hambrook and Sheath, 1987). In contrast to the other rhodophytes (*Batrachospermum virgatum*, *Tuomeya americana*) in this study, *A. violacea* had higher protein and lipid contents and the preferential grazing of this species is suggested to be due to the small, simple filaments and the relatively high protein content (Hambrook and Sheath (1987). Compared to our study, this investigation only analysed the grazing of different species of freshwater rhodophytes and not the grazing of different algal groups, like diatoms. Therefore it might be expected that grazers in the presence of other algal groups, avoid these rhodophytes due to the potentially higher nutritional quality of diatoms, for example. Current investigations aim to find out how algal groups (e.g. diatoms, chlorophytes, cyanophytes) differ from one another regarding their protein content and fatty acid composition (C. Winkelmann, personal communication).

Besides the content of nutrients and essential compounds, palpability might determine food quality of certain algae groups. The preference *Nitzschia* spp., *Surirella spiralis* and *Navicula cryptocephala* by the grazing mayfly *Ameletus validus* was also interpreted to be a consequence of their slight attachment to the substratum and therefore their good availability to the benthic grazers (Hill and Knight, 1987). We assumed that stalked diatoms such as *Gomphonema* sp would be more vulnerable to grazing because of their prominent growth forms than attached diatom species such as *Cocconeis placentula,* which is considered grazer-resistant because of their close adherence to the substrate (Moore, 1975; Gregory, 1983; Stevenson, 1996). This assumption was confirmed for *B. rhodani* and *Ecdyonurus* sp. which preferred stalk/tube forming diatoms. However, especially *E. ujhelyii* larvae preferred

attached/motile diatom species, such as *C. placentula*. The principle of different palatability was also suggested by Hambrook and Shelth (1987) to explain the preferential grazing of the rhodophyte *A. violacea* because this species has small and simple filaments. Following this line of thoughts, the avoidance of *Audouinella* sp. in the present study might not be linked to their nutritional value, but rather with the ability of heptageniids to feed on filamentous growth forms. Consequently, the failure of mayfly larvae to affect filamentous algae observed in this study might have been caused by a mismatch between mouthpart morphology and algal physiognomy. These different results indicate that previous assumptions might have been too general because different algal species may become more or less vulnerable depending on the ambient grazer species in a given stream.

Ecosystem implications and conclusions

Periphyton community in the experimental streams was mainly dominated by understory standing crop (diatoms) in addition to individual filamentous algae. If this is a general pattern for shaded, sub-mountain streams, grazers in such streams might be expected to be specialised on these dominating adnate and adhesive growth forms. Consequently it can be assumed, that the grazing pressure of the dominant grazer taxa in the studied streams was insufficient reduce filamentous algal growth forms to a considerable extent. Different grazer compositions adapted to the prevailing periphyton composition in other ecosystems might have other effects on the periphyton community. Therefore we conclude that a positive selection or specific reduction of filamentous algae would have been possible if other grazer species had dominated. Limnephilidae, Glossosomatidae (Trichoptera) and Prosobranchia (Mollusca) for example, are known to remove algal overstory in freshwater benthic ecosystems in most cases because caddisflies and snails might be better suited to remove the algal growth forms (Colletti et al., 1987; Steinman et al., 1987a).

Overall, our findings highlight the need of detailed analysis of the benthic grazing process, including consideration of feeding traits of particular benthic grazer communities, to understand the strength of trophic interactions between grazers and their primary food source, periphyton. Moreover, when looking at periphyton shaping effects, seasonal shifts in environmental conditions regarding the resource supply for benthic algae (e.g. nutrient and light availability) should be carefully considered (Lange et al., 2011; Winkelmann et al., 2014). Ultimately,

benthivorous fish have the potential to shape the composition of benthic algal communities via benthic grazers due to the occurrence of a top-down trophic cascade.

4 Sublethal effects of fish predation on benthic grazers

Evaluation of grazing activity and intensity of mayfly larvae during different levels of fish

4.1 Abstract

Flexibility is an important adaptive attribute of the feeding periodicity of grazing mayfly larvae because most natural environments offer a wide variation in local predation risk in terms of space, time or predator species.

In this study any changes of diel feeding periodicity and consumption rates of *Baetis rhodani* (Ephemeroptera: Baetidae) were analysed in response to different densities of benthivorous fish (*Barbatula barbatula* and *Gobio gobio*) by quantifying gut fullness using the fluorescence of algal pigments. Laboratory experiments with grazer species *B. rhodani* were conducted by using different concentrations of chemical fish cues. In order to assess the transferability of results to a larger scale, experimental results were compared with field observations in two second order streams using different densities of freely foraging benthivorous fish.

During the presence of chemical fish cues in the laboratory experiments the feeding periodicity of the *B. rhodani* larvae were mostly diurnal while in the absence of fish chemicals nocturnal feeding was observed. The same patterns could be detected in the field during the experiments with the different fish densities. These findings indicate that the larvae were able to assess variations in the predation risk and to alter their feeding habits by making flexible behavioural adjustments.

The results from the laboratory experiments further suggested that the behavioural response is controlled by fish density. Behavioural changes were observed for medium and high concentrations of the fish cues but not for a very low concentration. In the field however, the mere presence of fish seemed to be sufficient to induce the observed behavioural shifts. Although the presence of benthivorous fish seemed to cause a lower consumption rate of *B. rhodani* larvae in the field, such a reduction could not be found in the laboratory experiments.

A conclusion from this study is that the identification of behavioural modifications is an essential component needed for a better understanding of complex trophic interactions in benthic communities. Accurate evaluation and detailed observation of direct and indirect effects cannot be made without consideration of such behavioural mechanisms.

4.2 Introduction

Predation in stream ecosystems can have far-reaching effects on prey populations, either directly via predator-induced mortality and indirectly via sublethal effects such as behavioural adaptations (Peckarsky and McIntosh, 1998; Allan and Castillo, 2007a) or alterations of prey life-styles (Sih, 1987). The risk of mortality during the feeding process is an important influence on prey which causes them to alter their behaviour in order to reduce their vulnerability to predators (Sih, 1980; Holomuzki et al., 2010). One of the avoidance responses towards predators is to change activity times (e.g. Gentry, 1974; Nelson and Vance, 1979; Bertness et al., 1981). The benefit of this strategy clearly lies in the reduction of direct predation risk due to a decrease of time exposed to the predator. However, predator avoidance involves some costs, such as a reduction in time spent foraging, which makes it necessary to balance the potentially conflicting demands of maximising feeding rates and minimising predation risk (Sih, 1987). Therefore many prey species in streams facing benthivorous fish show trade-offs between foraging efficiency (energy intake) and predation risk (mortality) (e.g. Sih, 1980; Lima, 1985; Lima et al., 1985; Abrahams and Dill, 1989). Dahl and Greenberg (1996) and Dahl (1998b) additionally suggested that benthic-feeding predators might be more efficient at finding and capturing benthic prey than drift-feeding predators. It was suggested that they might have a more intense impact on benthic prey. However, experiments which have used exclusively benthivorous fish to determine the behavioural effects of mayflies are rare. Kohler and McPeek (1989) conducted a predation experiment with visually feeding benthic fish and observed strong behavioural effects of *Baetis* sp. (Ephemeroptera: Baetidae) during the day, when fish fed most actively. In a study with nocturnal benthic fish Culp et al. (1991) found two anti-predator responses of nocturnally active mayfly *Paraleptophlebia heteronea* (Ephemeroptera: Leptophlebiidae): movement into drift and retreat into interstitial crevices.

The majority of predation experiments were, however, conducted with larvae of *Baetis* sp. and with visually hunting and drift-feeding fish predators (mostly brown or brook trout, less frequently European minnow). They were focused mainly on the drift and exposure times of the larvae (e.g. Cowan and Peckarsky, 1994; Douglas et al., 1994). The higher predation risk caused by visually hunting fish during the day leads *Baetis* sp. larvae to retain their nocturnal periodicity (Peckarsky and McIntosh, 1998). In trout streams more larvae of *Baetis* sp. were found feeding on stone tops during the night than during the day (Cowan and Peckarsky, 1994). Several other studies have described an increased propensity for nocturnal drift of *Baetis* sp. larvae (e.g. Douglas et al., 1994; Tikkanen et al., 1994; Huhta et al., 1999; Miyasaka and Nakano, 2001; McIntosh et al., 2002), and a reduced exposure and drift activity during the daytime in the presence of visually feeding predators (e.g. McIntosh and Peckarsky, 1996; McIntosh and Peckarsky, 1999; McIntosh et al., 1999; McIntosh and Peckarsky, 2004). All the above mentioned predation experiments applied various approaches using either freely foraging fish or fish chemicals. However, a combined approach with different concentrations and densities of freely foraging benthivorous fish which integrates small and large spatial scales has still not been undertaken.

Flexible avoidance behaviour, linked to a specific predation threat, is an essential trait for most prey organisms. Moreover, it is a common and widespread solution (Lima and Dill, 1990). Its importance in benthic stream communities has been supported by many studies, which have shown flexible antipredator responses of stream insects to fish (e.g. Kohler and McPeek, 1989; Douglas et al., 1994; McIntosh and Townsend, 1994; Tikkanen et al., 1994; Tikkanen et al., 1996; McIntosh et al., 1999). Individual prey often show a pronounced flexibility with regard to their antipredator response, also known as short-term response, to predators (Sih, 1987), e.g. highlighted by the alterations to their activity schedules. Consequently, prey is able to adjust its behaviour to short-term changes in predator density perceived by predator cues (Douglas et al., 1994). For *Baetis* sp. chemical signals seem to be the most effective cue for predator detection (McIntosh and Peckarsky, 1999; McIntosh et al., 1999). Previous experiments have shown that *Baetis* sp. larvae are able to detect different concentrations of brook trout cues in stream water (McIntosh et al., 1999; McIntosh et al., 2002) and that chemical cues alone can cause an avoidance response (McIntosh and Peckarsky, 1999; Peckarsky et al., 2002; McIntosh and

Peckarsky, 2004). However, contradictory findings suggest that changes in drift rates of *Baetis* sp. were strongest during the direct presence of fish and that responses to live predators differed from responses to predator cues only (Tikkanen et al., 1994; Tikkanen et al., 1996).

Most experiments on predator avoidance behaviour of benthic invertebrates in streams are conducted for small temporal and spatial scales, probably because of practical reasons. Therefore such experiments cannot fully capture the consequences of predator avoidance behaviour on the ecosystem functions, due to experimentally necessary reductions in time, space and complexity (Carpenter et al., 1995; Petersen and Hastings, 2001). Thus, in such cases it is difficult to transfer obtained results to processes on the ecosystem scale (Carpenter et al., 1995; Schindler, 1998; Petersen and Hastings, 2001). In this work laboratory experiments were used to record the behavioural responses of mayflies to nocturnal feeding fish on the small scale and field observations were used to assess the possible effects of behavioural changes on a larger scale. The objective of this study was the identification of behavioural predator responses with regard to the feeding periodicity of *Baetis rhodani* (Ephemeroptera: Baetidae) under the influence of different concentrations of fish cues (stone loach: *Barbatula barbatula* and gudgeon: *Gobio gobio*) which was analysed in laboratory feeding experiments. The present study also included an evaluation of the consequences of predator avoidance behaviour on the general grazing intensity (consumption rate) and an assessment of transferability of predator effects on natural stream ecosystems. In order to achieve this goal an analysis was made of grazing activity and consumption rate of *B. rhodani* larvae in the field in two small mountain streams containing different densities of benthivorous fish over a three-year period. It was hypothesised that *B. rhodani* larvae will change their feeding periodicity pattern in order to avoid nocturnal feeding fish predators (**Hypothesis 2c**). Further, it was assumed that this predator avoidance behaviour will result in reduced grazing rates in natural stream ecosystems (**Hypothesis 2d**).

4.3 Materials and methods

Laboratory experiment setup

In the laboratory experiments the influence of chemical fish cues on feeding periodicity of *Baetis rhodani* larvae was investigated in April 2011. The experimental setup involved four treatments each with a different concentration of fish chemicals, which were replicated three times each: control (no fish cues), low, medium and high concentration. The trials were conducted in black circular stream tanks (Figure 4.1). In each experimental tank at least 40 *B. rhodani* larvae were introduced, after a measurement of their body length to the nearest 0.1 mm (TSO-VID-MESS-HY, Leica WILD M3C, Pulsnitz, Germany). Larvae had a mean body length of 9.5 ± 1.0 mm (± SD, n = 240) and were collected in the morning between 09:00 and 11:00 a.m. from the upstream fishless site of the treatment stream (see section s*tudy site*) and kept for 24 h in a glass tank with algae-covered rocks from the same site. Nymphs with black wing pads were excluded. These provided a food supply. Subsequently, the experiment was run for 48 h using a light regime of 12/12 hours (light/dark) which was similar to the natural conditions at the time of the year.

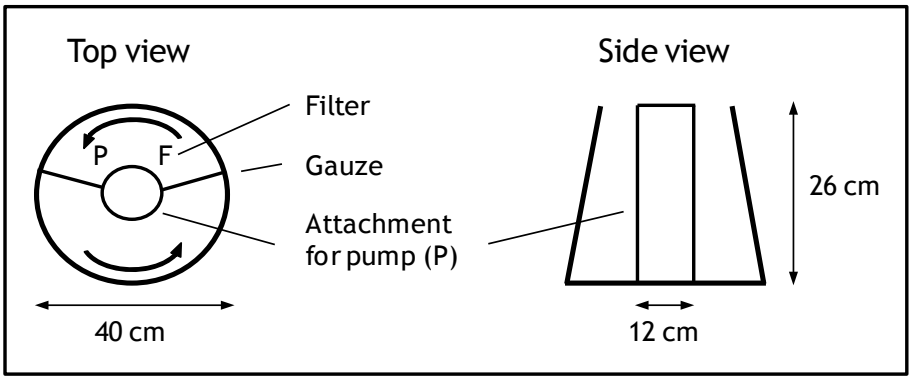

Figure 4.1 Design of the circular stream tanks used in the laboratory experiments (not to scale, P: Pump, F: Filter). Arrows in the top view indicate the flow direction.

Water containing chemical fish cues was taken from a 150 L aquarium including three fishes (this corresponded to a fish density of 200 Ind m^{-2}): one gudgeon (110 mm long) and two stone loaches (95 mm and 100 mm long), which were fed daily with frozen chironomids. On the basis of the average fish density of 0.6 ± 0.1 Ind m^{-2} (mean from 2007 to 2010 ± SD, n = 8) in the control stream (see

section s*tudy site*) with a mean of depth 0.1 m, a low (mean value divided by 10), a medium (corresponded to the mean value), and a high (mean value multiplied by 10) fish density was assigned. In order to reach a final water volume of 12 L in each experimental tank and to have the required fish density treatment, a calculated amount of fish-conditioned water from the aquarium was added to the fish-free water from the upper fishless site of the treatment stream. The experimental tanks were filled shortly before the experiment was started. The water temperature of 10.0 ± 0.1 °C (mean ± SD, n = 12) was similar to the natural stream temperatures at the time of year (9.3 ± 2.9 °C, mean ± SD, n = 13). In the circular tanks a mean current velocity near the bottom of 11.6 ± 4.9 cm s^{-1} (± SD, n = 12) was maintained with aquaria pumps (EHEIM, Aquarientechnologie, Deizisau, Germany). This value is between the natural velocity of pools (7.3 ± 3.5 cm s^{-1}) and riffles (35.5 ± 11.3 cm s^{-1}) in the control stream (mean ± SD, n = 20). All technical aquaria equipment (pumps and external filter) was separated from the experimental area by 1 mm mesh gauze (Figure 4.1).

During the trials, food and shelter for the larvae were provided by twelve unglazed and pre-cultured ceramic tiles (60 mm x 40 mm x 5 mm) per tank. The tiles were pre-cultured in a glass aquarium containing a homogenized and filtered (250 µm) periphyton suspension from the upper fishless site of the treatment stream. After 24 h of sedimentation in darkness the periphyton culture was incubated under natural light conditions for at least 14 days. During this time nutrients were added once a week (0.03 mg L^{-1} phosphorus as di-Natriumhydrogenphosphat, Na_2HPO_4) and the suspension was carefully ventilated with air stones and an external filter. A visible periphyton layer had developed on the upper surface of the tiles under spring light conditions after two to three weeks.

Laboratory sampling and analysis

The sampling of *B. rhodani* larvae started after a 24 h acclimatisation phase of larvae in the experimental tanks. Five larvae were sampled from each experimental tank every 4 h during a 24-hour-period. Larvae were placed individually in 2 mL Eppendorf tubes, immediately frozen in liquid nitrogen and, subsequently, stored there until further analysis.

Gut fullness of *B. rhodani* was measured fluorometrically (gut fluorescence analysis) as total pigment (TP, sum of chlorophyll *a* and phaeopigments) per dry

weight (dw) in general accordance with the method described in Cowan and Peckarsky (1990). However, gut pigments were extracted after freeze drying the larvae for 24 h (Lyophilisator Alpha 1-2, Martin Christ GmbH, Germany) and the determination of the body dry weight (to the nearest 0.1 mg dry mass) was made using a micro balance (B 120 S, Sartorius). Chlorophyll and phaeopigments were extracted in 90 % ethanol for 24 h at room temperature (Nusch and Palme, 1975). The fluorescence of the extracts was analysed in a Luminescence spectrometer (LS 50B, Perkin Elmer, Massachusetts, USA). In order to avoid an overestimation of the gut pigment contents, the average background fluorescence was analysed for 15 additional samples, which used the body of *B. rhodani* larvae without the guts. This background value (mean) was used to correct the measured values of gut fullness of *B. rhodani* larvae during the experiments in the laboratory and in the field.

Study site

The field study was conducted in two small second-order mountain streams (*Tännichtgrundbach* as control stream and *Gauernitzbach* as treatment stream), which flow into the River Elbe in eastern Germany (Saxony, Dresden, 51°06′ N, 13°32′ E, 120 m above sea level). The streams have characteristics of high substrate diversity and pronounced pool-riffle sequences.

Table 4.1 Physical and chemical characteristics (means ± 1 SE), the number of sampling occasions (*n*) and the significance levels (*p*) of environmental factors for the control and treatment streams during the study period 2008 to 2010. Italic values indicate significant differences (Welch two sample t-test).

Characteristics	Control	*n*	Treatment	*n*	*p*
Temperature (°C)	10.0 ± 0.6	51	9.6 ± 0.7	49	0.66
Oxygen saturation (%)	91.8 ± 2.0	48	90.9 ± 1.9	46	0.73
Oxygen concentration (mg L^{-1})	9.8 ± 0.3	48	9.7 ± 0.3	46	0.85
Electrical conductivity (µS cm^{-1})	570.1 ± 18.3	50	617.6 ± 18.2	49	0.07
Discharge (L s^{-1})	67.4 ± 8.0	48	39.7 ± 5.0	47	*0.004*
pH	7.9 ± 0.1	49	8.3 ± 0.0	50	*0.0002*
Phosphate (µg P L^{-1})	50.0 ± 4.3	31	34.2 ± 3.8	31	*0.007*

The upstream catchment areas of both streams are predominantly in agricultural use. Their lower areas, where the field observations and the sampling were performed, are dominated by deciduous woodland valleys (mainly *Betula*, *Acer*, *Fagus*, *Fraxinus* and *Quercus*). Due to the small width of the stream beds (1.5 to 2.3 m), the streams were almost completely shaded by the canopy of the riparian forest during the vegetation period. Both streams were similar with respect to their size, morphology and species composition (Schmidt et al., 2009b). However, the abiotic characteristics were slightly different between the two streams (Table 4.1). Observed differences in the range of discharge, pH and phosphate concentration were not expected to greatly influence the invertebrate community.

Study organisms

Table 4.2 Mean densities and biomasses, in dry weight (± 1 SE, 2008: n = 39, 2009: n = 30, 2010: n = 15), of total grazer community and of *B. rhodani* larvae over three generation cycles (2008 to 2010) in the control stream (Con), with high fish density, and in the treatment stream (Tre), with different manipulation scenarios (low, zero and high fish densities).

Year (Scenario)		**Density (Ind m^{-2})**		**Biomass (mg m^{-2})**	
		Grazer	*B. rhodani*	Grazer	*B. rhodani*
2008 (Low fish)					
	Con	312 ± 37	276 ± 33	51.0 ± 5.8	50.1 ± 6.5
	Tre	1025 ± 111	226 ± 29	199.9 ± 27.8	60.6 ± 12.9
2009 (Zero fish)					
	Con	275 ± 20	185 ± 16	66.3 ± 9.3	47.6 ± 9.3
	Tre	827 ± 92	188 ± 26	260.8 ± 63.8	48.0 ± 13.4
2010 (High fish)					
	Con	239 ± 45	107 ± 20	124.7 ± 54.3	40.7 ± 11.3
	Tre	239 ± 49	80 ± 8	71.5 ± 19.9	22.1 ± 6.1

Baetis rhodani (Pictet, Ephemeroptera: Baetidae) is a common grazer species, which often lives in the riffle habitats of streams (Elliott and Humpesch, 2010), and feeds

on algae and detritus attached to the upper surfaces of diverse substrates (Brown, 1961a; Bohle, 1978). This species is one of the most frequent grazers in the two studied streams, with a population ranging from between 22 to 88 % of the total grazer density (Table 4.2).

The vertebrate predators used in this study were the two small benthic stream fishes, stone loach (*Barbatula barbatula* (L.), Cobitidae) and gudgeon (*Gobio gobio* L., Cyprinidae), which are similar in size and ecology. During the three-year study period the total length of the gudgeon was about 91.9 ± 11.9 mm (mean ± SD, n = 1171) and that of the stone loach was about 105.8 ± 23.2 mm (mean ± SD, n = 743) in the two streams.

Field study design

In this study a pairwise ecosystem experiment was performed that facilitated analysis of trophic interactions between benthivorous fish, benthic invertebrates and algae (periphyton) by using different benthivorous fish stock in the studied two small streams. While the treatment stream (*Gauernitzbach*) contained different fish densities, the control stream (*Tännichtgrundbach*) was characterized by similar fish densities between subsequent years. The study areas in each stream were separated into two consecutive sections by fish barriers made of grids of high-grade steel meshes (5 mm mesh size). The downstream sections (400 m) of both streams were used as experimental areas for mayfly sampling. The upper sections (200 m) served as buffer areas for behavioural adaptation of any migrating mayflies coming from upstream sites to the fish manipulated environment. The sites upstream of the buffer areas were always kept fish-free in order to avoid any uncontrollable chemical cues from fish. Fish density was manipulated and monitored by undertaking electrofishing campaigns twice a year (every campaign contained two passes) in each stream over the whole period of the ecosystem experiment. For further explanation see Winkelmann et al. (2011).

Starting with a similar density of benthivorous fish in both streams, the fish density (*B. barbatula* and *G. gobio*) was experimentally reduced in the treatment stream by electrofishing from October 2006 to October 2009 but kept at a high level in the control stream. Thereby, in the study years 2008 and 2009 two different fish densities were contained in the treatment stream (low fish density in 2008 and 'zero' fish density in 2009), because fish elimination was conducted step by step, and the

fish density was still relatively high in spring 2007 (Table 4.3). In November 2009, fish were restocked in the treatment stream (high fish density) so that both streams contained benthivorous fish at similar densities (Table 4.3). This experimental arrangement followed the Before-After-Control-Impact (BACI) design (Stewart-Oaten et al., 1986), where differences between two ecosystems with different fish manipulation scenarios can be compared. In this study, the natural differences between both streams could be assessed in the year 2010 when there were high fish densities in the treatment and control streams.

In summary, the study was conducted over three generation cycles of *B. rhodani* where each had a different fish density in the treatment stream. In the control stream fish density was similar over the three mayfly generations during the study period (Table 4.3).

Table 4.3 Mean densities and biomasses, in fresh weight (n = 3), of fish stock over three generation cycles of *B. rhodani* (2008 to 2010) including spring 2007 in the control stream (Con), with high fish density, and in the treatment stream (Tre), with different manipulation scenarios (low, zero and high fish densities).

Year (Scenario)	Density (Ind m^{-2})		Biomass (g m^{-2})	
	Con	Tre	Con	Tre
Spring 2007	0.6	0.2	2.4	0.6
2008 (Low fish)	0.6	0.1	3.4	0.2
2009 (Zero fish)	0.6	< 0.01	4.0	< 0.1
2010 (High fish)	0.7	0.6	4.0	3.1

Field sampling and analysis

Physical and chemical water properties were measured every 28 days in the experimental areas of the two streams throughout the study period by using WTW probes (Weinheim, Germany, Oxi96, pH196 and LF340). Water temperature was monitored in 15-min intervals using a HOBO data logger (Onset, Massachusetts, USA). In order to quantify the phosphate concentration on each sampling occasion, 100 mL stream water was filtrated (cellulose acetate, 45 μm) and transported to the laboratory for analysis.

The invertebrate community was sampled every 28 days by using a Surber sampler (0.12 m^2, 500 μm meshes) from a randomly chosen pool/riffle sequence. Sampled invertebrates were rinsed over a 500 μm-sieve in the laboratory and stored in 80 % ethanol. Invertebrates of the benthic community were identified to the lowest possible taxonomic level. Body length (to the nearest 0.1 mm) and number of *B. rhodani* larvae were measured in each sample. The individual biomass of *B. rhodani* larvae (mg dry weight) was determined from a relationship between body length (BL) and dry weight calculated from 50 measured individuals: $weight = 0.0042 \times BL^{2.49}$. Other benthic invertebrates were mainly characterised using length-weight relationships (Meyer, 1989; Burgherr and Meyer, 1997; Benke et al., 1999).

Feeding periodicity of *B. rhodani* larvae was analysed on three sampling occasions in both streams, once during each different manipulation scenario (low fish: May 2008, zero fish: April 2009, high fish: May 2010). At least 15 larvae were sampled every 4 h over 24 h, starting 1 h after sunrise. For each sampling process samples were obtained from different riffles within the experimental area, starting at the lower end and moving upstream. Only larvae without black wing pads were collected, placed individually in 2 mL Eppendorf tubes, immediately frozen in liquid nitrogen and stored at -80 °C until the gut fluorescence analysis in the laboratory.

In order to analyse the foraging periodicity of benthivorous fish, at least 5 fish were sampled every 4 h during two 24-hour-campaigns in May 2004 (gudgeon, $n = 63$) and June 2005 (stone loach, $n = 30$) by electrofishing. Individuals were killed immediately, digestive tracts were removed and frozen as fast as possible and stored at -18 °C until further processing in the laboratory. The stomachs (stone loach) and anterior guts (gudgeon) were weighed to the nearest 0.1 mg of fresh mass, subsequently they were dissected, flushed and weighed empty. The stomach fullness was calculated from the ratio of food mass to fish mass ($g\ g^{-1}$).

Data analysis

All experimental results of gut fullness (*B. rhodani*) and stomach fullness (stone loach and gudgeon) were expressed as relative values. For each sampling occasion or experimental trial the relative gut/stomach fullness was calculated in relation to absolute values of the maximum gut or stomach fullness.

The daily individual consumption rate (*C*) per mg dry weight (µg TP mg^{-1} dw) of *B. rhodani* larvae was calculated directly from gut fullness data obtained during the 24-hour-sampling periods (field or laboratory) in accordance with the method of Elliott and Persson (1978). Exponential evacuation rates (*R*) were derived from the maximal rate of decline in gut fullness throughout each 24-hour-period (Madon and Culver, 1993). For the time interval, which represented this decline in gut fullness, a linear function was fitted to the logarithms of the values of gut fullness, with y = ln (gut fullness) and x = time interval (4 h). A general gut evacuation rate was calculated for the two streams (control and treatment streams 2008 to 2010) and for the laboratory experiment (4 treatments) by averaging the single evacuation rates estimated for each sampling occasion or experimental trial (Table 4.4).

Table 4.4 Measurement parameters for estimation of daily individual consumption of *B. rhodani* larvae. Time periods of maximal decline in gut fullness and linear functions fitted to these intervals in the four treatments regimes of the laboratory experiment, in the control stream (high fish density), and in the treatment stream (different manipulation scenarios: low, zero and high fish densities). Italic values indicate the gut evacuation rates (ng TP mg^{-1} dw h^{-1}).

	Treatment/Scenario	Time of day	Linear function
Laboratory experiment	Control	05:00 to 09:00	y = *-0.3186*x + 4.2571
	Low	13:00 to 17:00	y = *-0.2425*x + 4.6406
	Medium	13:00 to 17:00	y = *-0.0192*x + 4.7442
	High	13:00 to 17:00	y = *-0.1082*x + 4.4628
Control stream	May 2008	19:00 to 23:00	y = *-0.0992*x + 5.6734
	April 2009	19:00 to 23:00	y = *-0.1691*x + 6.1035
	May 2010	11:00 to 15:00	y = *-0.1732*x + 6.3574
Treatment stream	Low fish	19:00 to 23:00	y = *-0.5556*x + 6.3174
	Zero fish	07:00 to 11:00	y = *-0.2508*x + 6.0513
	High fish	11:00 to 15:00	y = *-0.1667*x + 5.9664

The monthly individual consumption rate was calculated from the daily consumption rate multiplied by the number of days per month (30 or 31 days). In order to estimate the total consumption of the *B. rhodani* population in each sampling month (mg TP m^{-2}) in the control and treatment streams, the individual value was multiplied by the biomass of *B. rhodani* (mg dw m^{-2}).

The differences in feeding periodicity of *B. rhodani*, obtained in the various laboratory treatments, were tested with a two-way ANOVA (factors 'fish chemical cues' and 'time') and following multiple comparison test (Tukey-HSD test with Bonferroni correction). For the 'fish chemical cues' the following levels were defined: control (no fish cues), low, medium and high concentration of fish cues, whereas only two levels were defined for the factor 'time' (day vs. night). Therefore all values of gut contents obtained at 13:00 were included in the day-values because this sampling point represented the middle of the light period. Whereas all values obtained at 01:00 were defined as night-values because they represented the middle of the dark period within our experimental light regime. The difference in the daily individual consumption rates of *B. rhodani* between the treatments in the laboratory experiment were tested with a one-way ANOVA with 'fish chemical cues' as the factor. The influence of fish on *B. rhodani* biomass in the field was assessed using Randomised Intervention Analysis (RIA) as described in Carpenter et al. (1989). Consequently, the mean differences of *B. rhodani* biomass between the treatment stream and the control stream (riffle areas) for each fish manipulation scenario were calculated. In addition two separate comparisons were also made: zero fish vs. low fish and zero fish vs. high fish using a randomisation test (4000 iterations).

SigmaPlot (Systat Software Inc., Version 11.0) was used to fit the linear evacuation rates. All other statistics were calculated using the software R (R Development Core Team, Version 2.15.0, 2012).

4.4 Results

A dominance of nocturnal feeding periodicity of *Baetis rhodani* larvae was observed in the control (without fish chemical cues) of laboratory experiments (Figure 4.2a) because gut fullness was significantly higher at night than during the day ($p = 0.005$, $n = 3$, Tukey-HSD test). The dominance of day time feeding regime was observed in the experimental treatments containing high and intermediate concentrations of fish chemicals (Figure 4.2b) because gut fullness was higher during the day than at night

(p = 0.63 and p = 0.32, n = 3, Tukey-HSD test). Feeding periodicity was weak in the experimental treatment with low fish cue concentration, where *B. rhodani* fed similarly during the day and night (p = 1.0, n = 3, Tukey-HSD test, Figure 4.2b). While no overall difference in the gut fullness was found between the various treatments (ANOVA; F = 0.58, p = 0.63), feeding periodicity appeared to depend on the concentrations of fish chemicals. Significant differences were found for the gut fullness at night between the control, and the treatment with high fish cue concentration (p = 0.049, n = 3, Tukey-HSD test) and the treatment with intermediate fish cue concentration (p = 0.008, n = 3, Tukey-HSD test), but not to the treatment with low fish cue concentration (p = 0.2, n = 3, Tukey-HSD test). The gut fullness during the day did not show any significant differences between the various experimental treatments.

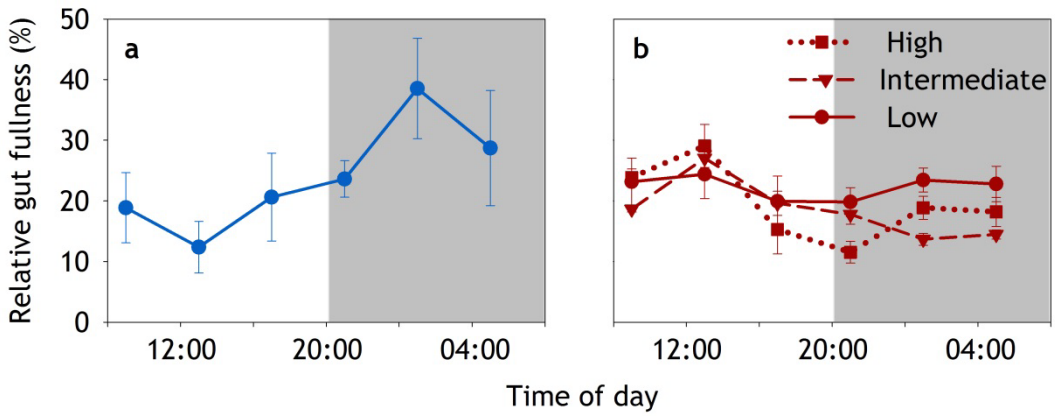

Figure 4.2 Relative gut fullness (mean ± 1 SE, n = 15 animals per data point) of *B. rhodani* larvae over 24 h in the laboratory experiment: (a) treatment without fish chemicals (control), and (b) treatments with different concentrations of fish chemicals. Grey areas mark the time of darkness during the experiment.

In their natural environment *B. rhodani* were observed to feed mostly during the day in the control stream where a high density of benthivorous fish were present in all the years of the study (Figure 4.3). Similar patterns of feeding activity were observed in the treatment stream with low fish density (2008) and high fish density (2010) (Figure 4.3a and c), although the fish density in the treatment stream varied considerably between these years (Table 4.3). By averaging the relative gut fullness of both years (2008 and 2010) for day and night, gut fullness was observed to be

lowest during the night (control stream: 28 %, treatment stream: 26 %) and highest during the day (control stream: 43 %, treatment stream: 38 %). In 2009, when no fish were in the treatment stream, the feeding activity of *B. rhodani* larvae was different from the pattern in the control stream (Figure 4.3b). In fact, feeding periodicity appeared to be reversed because relative gut fullness increased steadily from the evening, continued to increase during the night and reached a maximum of 50 % in the early morning (Figure 4.3b). At the same time in the control stream, mainly daytime feeding was measured, similar to the patterns observed in this stream in the years 2008 and 2010 (Figure 4.3).

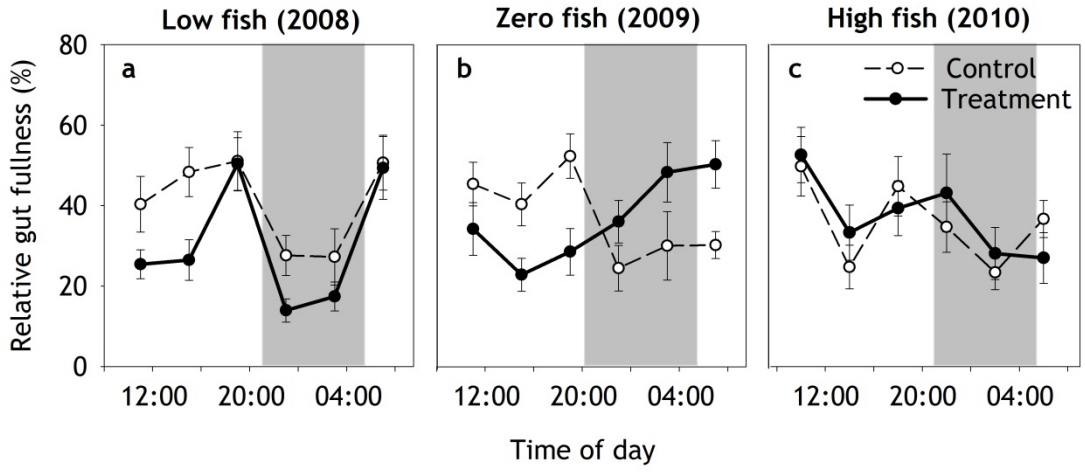

Figure 4.3 Relative gut fullness (mean ± 1 SE, n = 15 animals per data point) of *B. rhodani* larvae over 24 h in the control stream (high fish density) and the treatment stream (different manipulation scenarios) in the months when samples were taken: (a) low fish (May 2008), (b) zero fish (April 2009) and (c) high fish (May 2010) density. Grey areas mark the time between sunset and sunrise.

In addition, the feeding periodicity of *B. rhodani* larvae in the treatment stream for the 'zero fish scenario' was similar to the pattern observed in the laboratory experiment in the control treatment without chemical fish cues (Figure 4.2a and Figure 4.3b). Both fishes, gudgeon and stone loach, generally showed a nocturnal feeding periodicity in their natural environment. For gudgeon we observed the highest stomach fullness during the night and for stone loach at dusk (Figure 4.4).

The mean gut evacuation rates of *B. rhodani* mayfly larvae, calculated from the values of all individual experiments (Table 4.4), were in the laboratory experiment

R = 0.130 µg TP mg^{-1} dw h^{-1} and in the field study R = 0.236 µg TP mg^{-1} dw h^{-1}. Daily individual consumption rates did not differ between the different fish cue treatments in the laboratory experiment (ANOVA; F = 0.63, p = 0.62) (Figure 4.5a). In the field study, daily individual consumption seemed to be generally higher in the control stream than in the treatment stream (Figure 4.5b). However, the consumption rate in the treatment stream for the 'zero fish scenario' (2009) relative to the control stream appears to have increased compared to the other fish scenarios (Figure 4.5b) because the daily individual consumption rate in the treatment stream was very similar to the control stream with only a difference of 5 %. In contrast to this, the daily consumption rates in the treatment stream for the 'high fish scenario' and for the 'low fish scenario' were 26 - 49 % lower than in the control stream.

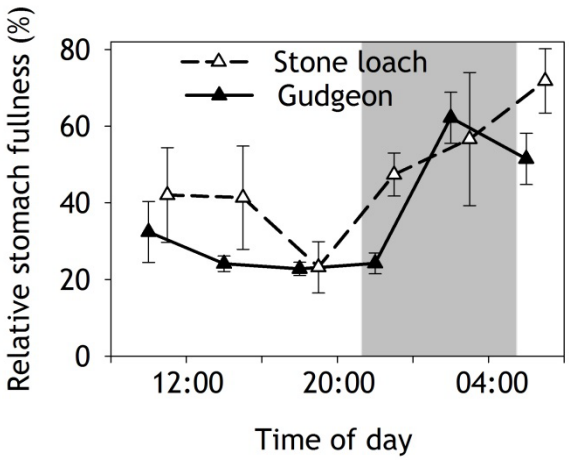

Figure 4.4 Relative stomach fullness (mean ± 1 SE) of stone loach in June 2005 (n = 5 animals per data point) and gudgeon in May 2004 (n = 10 animals per data point) over 24 h in the treatment stream. Grey area marks the time between sunset and sunrise.

Larger effects are possible on the ecosystem scale as an estimation of the total consumption of *B. rhodani* in the two studied streams revealed. While for low and high fish densities in the treatment stream there was a lower total consumption than in the control stream, total consumption was considerably higher in the absence of benthivorous fish (zero fish, 2009, Table 4.5). This might be caused by a combination of changes in the daily consumption rate and benthic biomass of *B. rhodani* larvae in the field, although no statistically significant differences could be shown for either

factor (daily consumption rates in the field without replicates; biomass zero vs. high fish: p = 0.45, biomass zero vs. low fish: p = 0.82, randomisation test, 4000 iterations, n_{low} = 14, n_{zero} = 12, n_{high} = 9).

Table 4.5 Estimation of total consumption (mg TP m^{-2}) of *B. rhodani* population in the control stream (high fish density), and in the treatment stream (different manipulation scenarios: low, zero and high fish densities).

Year (Scenario)	Control stream	Treatment stream
2008 (Low fish)	6.75	2.55
2009 (Zero fish)	3.05	12.25
2010 (High fish)	2.49	1.01

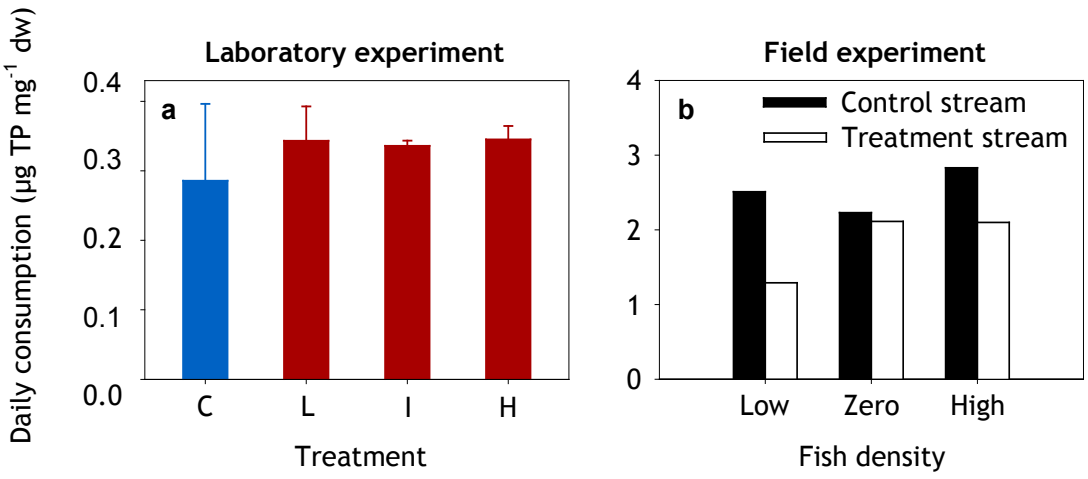

Figure 4.5 Daily individual consumption of *B. rhodani* larvae: (a) for the four treatment regimes (C: Control, L: Low, I: Intermediate, H: High) in the laboratory experiments and (b) in the control stream (high fish density) and the treatment stream with different manipulation scenarios: low fish (2008), zero fish (2009) and high fish (2010) densities. The consumption rates in the laboratory experiments were estimated by using five samples per interval over a 24-hour-cycle for three replicates (mean ± 1 SE, n = 3) whereas the consumption rates in the field experiment were estimated only once per year by taking 15 samples per interval over a 24-hour-cycle in each sampling month of the study period.

It should be noted that the average daily individual consumption rate of *B. rhodani* larvae in the laboratory experiment was only 15 % of the average specific consumption in the field, largely because of the measured lower absolute gut fullness in each treatment (Table 4.6).

Table 4.6 Absolute gut fullness (means ± 1 SE) of *B. rhodani* larvae and number of measured individuals (*n*) for the four treatment regimes of the laboratory experiments, in the control stream (high fish density) and in the treatment stream (different fish scenarios).

	Treatment/Period	**Gut fullness** (ng TP mg^{-1} dw)	***n***
Laboratory experiment	Control	92.0 ± 10.3	88
	Low	109.9 ± 12.2	89
	Medium	108.1 ± 7.8	86
	High	111.5 ± 10.4	89
Control stream	2008 to 2010	360.5 ± 16.1	357
Treatment stream	2008 to 2010	302.0 ± 14.2	347

4.5 Discussion

The behavioural responses of benthic invertebrates to predation risk have been observed before, at least for visual hunting diurnal active fish (e.g. Bechara and Moreau, 1992; McIntosh and Townsend, 1996; Dahl, 1998a; Muotka et al., 1999; Herbst et al., 2009). For the case of *Baetis* sp. larvae there are many studies which describe the behavioural responses used to avoid predation by diurnal fish (e.g. Kohler, 1984; Cowan and Peckarsky, 1994; McIntosh and Peckarsky, 1996; Huhta et al., 1999; McIntosh and Peckarsky, 1999). In all of these studies *Baetis* sp. larvae become more nocturnal in their feeding activity when exposed to diurnal active and visually hunting fish. In this study it was hypothesised that in the presence of nocturnal benthic feeding fish *Baetis rhodani* would similarly change their temporal feeding periodicity in order to avoid this predator type (**Hypothesis 2c**), and that the intensity of the response would depend on the fish density. It was further assumed that as a consequence of this predator avoidance behaviour, grazing rates in

natural stream ecosystems would be reduced due to the presence of fish (**Hypothesis 2d**).

The first hypothesis (**Hypothesis 2c**) was clearly supported by the results of the laboratory experiments and the field observations. This indicates the relevance of these findings to natural stream ecosystems. In the laboratory and in the field study, similar behavioural patterns, namely a nocturnal feeding periodicity, could be observed in the absence of fish cues or fish. In both studies, an inverse feeding pattern was observed when fish or fish cues were present. Benthivorous fish, stone loach and gudgeon, seemed to pose the highest risk to the *B. rhodani* larvae during the night (Worischka et al., 2012). This resulted in a shift of their temporal feeding pattern from nocturnal to diurnal. Such a shift in the presence of fish or its cues was interpreted as predator avoidance behaviour of the *B. rhodani* larvae. The results from the laboratory experiments further suggested that this behavioural response was related to the intensity of predation threat because there seemed to be a transition from nocturnal feeding in the absence of fish cues to indifferent feeding at low fish cue concentration to strictly diurnal feeding for high fish cue concentrations. This reaction seems a reasonable route to balance benefits (effectively avoiding fish) and costs (feeding efficiently) of the predator avoidance behaviour. This point of view is supported by a study of Sih (1982) in which he also found that the degree of avoidance behaviour is proportional to the magnitude of the predation risk to be cost-effective. However, in the direct presence of fish in the streams, *B. rhodani* larvae were observed to alter their feeding periodicity even for very low fish densities. The low fish density in the treatment stream should have resulted in a fish cue concentration similar to those of the 'low fish treatment' in the laboratory experiment. Tikkanen et al. (1994; 1996) found that while fish cues alone can trigger an avoidance response, additional hydrodynamic and/or visual cues from living fish are perceived as an increased predation risk. It has even been suggested that prey animals should be able to assess the predation threat from any available predator cues in order to maximise their fitness (Sih et al., 1998). Miyasaka and Nakano (2001) also concluded that the use of cues for the process of predator-avoidance varied with the type of predatory fish because of differences in the availability of such cues to the prey invertebrates. Hence, flexible and rapid avoidance behaviour appropriate to the specific predation threat is an essential trait needed for most prey organisms in order to effectively reduce encounters with predators (Lima and Dill, 1990), because

being able to assess the risk of predation and to respond to the presence of a predator before the predator detects the prey is a major benefit to prey (Kohler and McPeek, 1989).

Several previous studies reported a nocturnal activity peak of *Baetis* sp. under natural conditions (e.g. Elliott et al., 1988; Glozier and Culp, 1989; Cowan and Peckarsky, 1994; McIntosh and Townsend, 1994; McIntosh and Peckarsky, 1996). Nocturnal activity is generally assumed to be a predator-avoidance mechanism which enable a reduction in encounters with visually hunting predators such as trout (Allan, 1983; Sih, 1987). However, in the predator-prey combination investigated in this study, the fish did not hunt visually and caused the highest predation threat during the night. Consequently, a temporal pattern inverse to those reported in literatures seems logical. As predation risk is known to affect the diel feeding periodicity in many stream invertebrates (Culp et al., 1991; Cowan and Peckarsky, 1994; Tikkanen et al., 1994) it is assumed that prey is generally most active when the predators are inactive in order to reduce the risk of predation (Sih, 1985; Sih, 1987). This is supported by the observations of a diurnal activity pattern as a response to nocturnal fish predators in this study. This indicates that the predator avoidance behaviour of *B. rhodani* larvae is highly adaptive, which enables this species to respond accordingly to the foraging pattern of the relevant predatory fish. Thereby, larvae are expected to benefit from decreasing encounter rates with benthivorous fish and associated decreasing risk of death. This line of argument is supported by the fact that not only the presence, or absence of fish, but also the fish species can affect the behavioural responses of mayflies (McIntosh, 1995; McIntosh and Peckarsky, 2004). Hence, the findings of this work support a general prediction of (Allan, 1983) that stream invertebrates have a broad spectrum of effective behavioural or morphological adaptations which are used to avoid fish predators.

In the stream ecosystems studied in this work, *B. rhodani* larvae are confronted with the following conflict: the best feeding places pose the greatest risk to mortality due to the nocturnal habitat overlap with the fish predators. Some authors have shown that prey use low quality feeding sites in the presence of predators (e.g. Stein and Magnuson, 1976; Sih, 1982; Werner et al., 1983; Power et al., 1985). A prey`s optimal behaviour should be to maximise its fitness and to reduce its mortality risk at the same time (Dill, 1987). Therefore prey behaviour is expected to be a trade-off between predator avoidance and fitness maximisation (Lima and Dill, 1990).

Predator avoidance behaviour generally involves energetic costs (Dill, 1987). In addition, the associated reduction in time spent foraging can be expected to decrease the food consumption. Therefore lower consumption rates might be expected to be a consequence of the avoidance response of *B. rhodani*. The field observations seemed to concur with this because when there were no fish, the individual consumption of *B. rhodani* larvae was higher than when higher fish densities were present. This difference was even more pronounced for the estimated consumption by the whole population. Therefore benthic grazing might be reduced in a risky environment involving fish predators by a combination of behavioural and density effects. The field data indicated that in addition to benthic density and community composition, behavioural changes should also be taken into account when the effects of predation on ecosystem functions such as grazing, are considered. In regard to the second hypothesis (**Hypothesis 2d**), however, there was no evidence for a fish effect on grazing rates due to the lack of replication on the ecosystem scale. This is compounded by there being observations of any reduction of daily individual consumption rate due to the fish chemicals in the laboratory experiments. The results of previous studies, on the other hand, show reductions of food intake and changes in algal consumption as a result of predator avoidance behaviour (Culp et al., 1991; McIntosh, 1995). The differences between the experiment presented here and those studies might be explained by the exceptionally early emergence of *B. rhodani* larvae in April 2011 in this study. Typically, in other years the emergence of *B. rhodani* occurred between May and June. In 2011 however, observations were made of some larvae developing black wing pads (indicating immanent emergence) already during the experimental time in April. This was probably caused by the relatively warm spring in this year. Consequently, it was suspected that most of the larvae used in the laboratory experiments may have developed into very old larval instars and begun to reduce food intake due to the associated reduction in guts and mouthparts (Williams and Feltmate, 1992; Wichard et al., 2002). This view may be supported by the fact that the mean consumption rates in the laboratory experiments were about three times lower than that measured in the field. Therefore the inability of this work to show a reduction of consumption rate as a consequence of predator avoidance behaviour may be due to experimental difficulties and does not necessarily mean that no reduction of grazing rate can be expected to occur in natural stream ecosystems.

In conclusion, feeding periodicity of *B. rhodani* varies according to the predator regime (fish vs. fishless). This was evident in both small scale laboratory experiments and on the ecosystem scale. *B. rhodani* larvae were found to be highly sensitive to chemical cues related to the actual predation threat of nocturnal benthivorous fish. Depending on the actual risk of predation from benthivorous fish *B. rhodani* responded rapidly by showing behavioural flexibility. However, further investigations are necessary in order to verify if there are any associated reductions of consumption rates and to quantify possible fitness consequences for *B. rhodani* in natural stream ecosystems.

5 Synthesis

5.1 Evaluation of preconditions for biomanipulation

The control of internal ecological processes (e.g. ecotechnology) in combination with reduction of external nutrient loads is known as additional measure to effectively minimise symptoms of freshwater eutrophication (Benndorf, 1990). The principles and processes involved in modifying the food web structure by biomanipulation are well understood in pelagic food-webs (e.g. Benndorf et al., 1988; Brett and Goldman, 1996; Benndorf et al., 2002). It is intended that the ecosystem service 'benthic grazing' (periphyton consumption) might be used as ecotechnological tool for periphyton control in order to reduce heavy algal growth and mitigate eutrophication effects in running waters. This thesis analysed the effects of fish predation on grazer-periphyton interaction in small streams. The objective was to assess mechanisms and processes such as

- generally, the possibility of top-down control on algal biomass by benthivorous fish,
- specific effects of benthic grazers on periphyton community composition during fish presence/absence and
- predator avoidance by benthic grazers.

All these mechanisms are expected to contribute to the success of stream food web manipulations via benthivorous fish, and consequently, represent a small selection of substantial preconditions for feasibility of the biomanipulation concept with the objective of a potential eutrophication control in running water ecosystems.

Benthivorous fish (stone loach and gudgeon) induced a trophic cascade via benthic grazers to periphyton: fish reduced grazer biomass and indirectly increased periphyton biomass. T**op-down control on periphyton** (Chapter 2) varied seasonally in temporally shaded streams in deciduous woodland valleys as indicated by dynamic modeling. Top-down control on periphyton was strongest during autumn and early winter when periphyton accrual was light-limited due to shading by the riparian canopy, whereas bottom-up control of periphyton was strongest in spring. At that time periphyton accrual is not light-limited due to still missing foliation.

Benthivorous fish can indirectly affect the **periphyton community composition** (Chapter 3). This grazer-mediated alteration was caused by a combination of

selective feeding by the mayfly grazers and changes in grazer biomass. Diatoms generally benefited from the presence of benthivorous fish due to biomass decrease of grazer species showing a strong preference for diatoms. Despite reduction of total grazer biomass two grazers (Ephemeroptera: *Baetis rhodani*, *Ecdyonurus* sp.), which preferred the group of stalk/tube forming diatoms, increased their biomass during fish presence in relation to other grazer species. The grazing pressure on stalk/tube forming diatoms also increased resulting in a heavy reduction of this diatom fraction during fish presence. We did not observe a general structural alteration of periphyton physiognomy by the performance of benthic mayfly grazers. The studied mayfly species were not able to remove algal overstory and establish an adherent algal understory canopy because all four mayfly species avoided filamentous algal species and benthivorous fish did not affect dietary preferences of mayfly grazers.

Predator avoidance behaviour (Chapter 4) was evident for the mayfly species *B. rhodani* (Ephemeroptera: Baetidae) which showed a shift of their temporal feeding pattern from nocturnal to diurnal in the presence fish or its cues. The feeding periodicity of mayfly larvae responded inverse to their benthivorous fish predators, stone loach and gudgeon, because these fish species generally showed a nocturnal feeding pattern in their natural environment. This behavioural response was related to the intensity of predation threat indicating that *B. rhodani* larvae were highly sensitive to fish chemicals. Consequently, larvae were able to change their feeding habits by making flexible behavioural adjustments depending on the actual risk of predation. An associated reduction of grazing rates could not ultimately be demonstrated.

Evaluating the importance of analysed preconditions for a potential implementation of biomanipulation in running waters, the existence of a top-down trophic cascade under natural conditions is expected to be most important. The three-level trophic cascade from benthivorous fish through benthic grazers to stream periphyton provides the basis for any application of the biomanipulation approach in running waters because predation is the driving force in structuring of food-webs (Krivan and Schmitz, 2004). Periphyton can be top-down controlled by benthic grazing if grazing pressure is combined with light limitation as was demonstrated in the present study.

Besides general reduction of periphyton biomass the structural change of periphyton community, i.e. removing algal overstory and establishing an adherent algal understory, is also an important objective of a successful biomanipulation. The

ability to control filamentous algae is expected to reduce clogging (colmation) of the hyporheic zone and facilitate water exchange in eutrophic stream ecosystems (Ibisch et al., 2009). However, the attempt to structurally change the periphyton matrix failed because studied mayfly species negatively selected filamentous algal growth forms. This seems reasonable as diatoms are generally more palatable and have higher nutritional values than cyanophytes or filamentous chlorophytes (Gregory, 1983; Hart, 1985; Rosemond, 1993; Rosemond et al., 2000).

In the present study, periphyton community consisted of a small proportion of filamentous chlorophytes and cyanophytes, while filamentous rhodophytes (*Audouinella* sp.) formed the major part of filamentous algal species in the streams. Other than cyanophytes and filamentous chlorophytes, species of rhodophytes especially *Audouinella* sp. seem to be of higher nutritional quality (high protein and lipid content) and can be used intensively by grazing mayflies (Hambrook and Sheath, 1987). In contrast to our study, Hambrook and Sheath analysed the grazing of different freshwater rhodophytes (e.g. *Audouinella violacea*, *Tuomeya Americana, Batrachospermum virgatum*) only and did not compare the outcome with the grazing of other algal groups, like diatoms. Therefore it might be expected that grazers in the presence of different algal groups, prefer other algal species than rhodophytes due to their potentially higher nutritional quality. Current investigations aim to determine how algal groups (e.g. diatoms, chlorophytes, cyanophytes) differ from one another regarding their protein content and fatty acid composition (C. Winkelmann, personal communication). In the present study it is very likely that avoidance of *Audouinella* sp. has been caused by missing ability of grazers to feed on these filamentous growth forms, although *B. rhodani* were generally capable of using filamentous algae such as rhodophytes.

Feeding behaviour of benthivorous fish and avoidance behaviour of benthic grazers are further important mechanisms for cascading effects because top predators thereby can influence lower trophic levels indirectly. These indirect interactions often have the potential to mask and overwrite direct effects and thus complicate the interpretation or prediction of community and food-web dynamics (e.g. Menge, 1995; Abrams et al., 1996; McCann et al., 1998). The change of periphyton biomass by benthivorous fish (indirect effect) in the studied food web might have been caused either by density-mediated or trait-mediated indirect interactions or by a combination of both indirect interactions. A density-mediated indirect interaction

occurred when benthivorous fish regulated the abundance of benthic grazers resulting in biomass changes of periphyton. A trait-mediated indirect interaction (sublethal effect) might be assumed for the species *B. rhodani* due to modifications in their feeding behaviour from initially nocturnal to diurnal, according to the feeding behaviour of benthivorous fish. In conclusion no clear evidence for an effect on periphyton biomass due to reduced grazing rates could be found. For benthic grazers, like mayfly larvae, such behavioural response can result in reduced food intake (Sih, 1980; Culp et al., 1991; McIntosh, 1995; Holomuzki et al., 2010) which could have effects on their growth rates, body size, fecundity, and finally, fitness consequences for the prey (Allan and Castillo, 2007a). Consequently, a change in foraging behaviour resulting in starvation would also reduce densities of the prey populations long-term (McNamara and Houston, 1987; McNamara and Houston, 1996). In other words, trait-mediated indirect interactions can have similar directional effects than density-mediated indirect interactions. The differentiation between both interaction types seems to be challenging in both natural and artificial systems due to their cross masking (Krivan and Schmitz, 2004) and was not intended here. In addition, trait-mediated indirect interactions are assumed to have even stronger impacts on food webs than density-mediated effects (Krivan and Schmitz, 2004).

Certain mayfly species (Ephemeroptera: *Baetis bicaudatus*) showed differential avoidance responses in the presence of fish and invertebrate predators (Peckarsky and McIntosh, 1998). This indicates that the nature of indirect effects seems to be related to the specific predator type which is present in the relevant system. Based on their hunting mode and their habitat, different predator species induce different avoidance responses in a prey species (Schmitz et al., 2004). Hence, multiple predators are able to alter the extent and spatial distribution of prey effects on their algal food resources. The food web of interest in the present study offered a diverse predator community such as two species of benthivorous fish and several invertebrate predators. The two benthivorous fish species were chosen to ensure predation pressure on both main habitat types; while stone loach mostly hunts in riffles, and gudgeon mainly preys in pools (Zweimüller, 1995; Mastrorillo et al., 1996; Prenda et al., 1997; Erös et al., 2003; Worischka et al., 2012).

Predation pressure in the absence of benthivorous fish was at least partly compensated by invertebrate predators in the studied streams (Hellmann, 2010). However, grazing mayfly larvae (Baetidae, Heptageniidae) as key stone species for

biomanipulation in the small streams remained largely unaffected by invertebrate predators, whereas species of the orders Diptera and Plecoptera were consumed to a greater extend. Hence, the importance of invertebrate predation seems not to be relevant for the biomanipulation approach in the studied streams (Hellmann, 2010), although invertebrate predators should always be considered within a stream food web.

Temporal variation in the strength of top-down control on periphyton was estimated using a dynamic model which indicated that light supply and its seasonal change has attenuated top-down control on periphyton within the studied food web. In the streams studied, phases of effective top-down control on periphyton alternate with phases of bottom-up control. Temperate ecosystems are driven by seasonal changes in temperature, light and cyclical growth phases, e.g. leaf-out in spring, leave fall in autumn associated with the deciduous phenology of the dominant riparian trees (Sandvik et al., 2002). This seasonal variation affects periphyton and can modify direct and indirect trophic interactions (Rosemond et al., 2000; Sandvik et al., 2002; Kishi et al., 2005; Buria et al., 2010). In the present study for example, seasonal shifts in the environmental factors temperature and light supply caused a decreased intensity of periphyton top-down control due to changes in biological rates such as benthic grazing and primary production.

The seasonal availability of periphyton is obvious, especially in early spring when periphyton biomass reaches its maximum due to the high light supply at this time of the year. Because most benthic algae are able to grow rapidly and usually have short generation times of a few days only (Lamberti, 1996; John et al., 2002), they can react quickly to seasonal shifts in resource availability. In contrast, the biomass of benthic grazers is relatively stable resulting in more or less constant grazing rates (Bauernfeind and Humpesch, 2001). Therefore a short-term biomass increase of benthic grazers during growing food supply, similar to the one observed for *Daphnia* sp. in standing waters, is not possible in running waters.

The periphyton community composition itself also shows seasonal variations, for example diatoms tend to be dominant during spring, whereas filamentous cyanobacteria and/or green algae are dominant end of summer (Moore, 1977). This shows that the diet of benthic grazers can vary due to the availability of various algal species but also due to location of food and developmental stage larvae (Lamberti and Moore, 1984; Williams and Feltmate, 1992). Despite these variations,

benthivorous fish were able to induce alterations of periphyton community composition via grazing mayflies in the present study. This change resulted from the combination of two different mechanisms: studied mayfly species fed selectively on diatoms, especially species located in the lowest and middle layer of periphyton matrix and alterations in grazer biomass resulted in an over-use or an under-use of this taxon.

From the information above it is evident that several of the examined mechanisms and processes represent substantial preconditions for a prospective transfer of the biomanipulation concept to running water ecosystems. There is additional evidence that these processes are linked to each other and interact in many ways shaping trophic interactions in stream food webs. Consequently, the regulation of periphyton growth in small temperate mountain streams is a complex interaction of top-down and bottom-up forces and seems to be possible only in a certain time frame when top-down (grazing) and bottom-up effects (light limitation) occur in combination. This observation is supported by Rosemond et al. (1993) who stated that dual control of periphyton biomass by consumers and resources are more important than top-down and bottom-up control alone. It can be expected that only a combination of external and internal mechanisms will lead to a stable improvement in water quality of aquatic ecosystems (Benndorf and Kamjunke, 1999). Nevertheless research on biomanipulation substantially contributes to understanding trophic interactions in complex stream food webs which in turn can promote the potential success of the biomanipulation approach in running waters. It can be concluded that biomanipulation generally appears possible but its feasibility is restricted to very small streams with deciduous bank vegetation. The assessment of preconditions for biomanipulation supported several but not all hypotheses proposed in chapter 1:

- **Hypothesis 1** was clearly supported because periphyton biomass in streams could be top-down controlled by benthivorous fish via benthic grazers.

- The presence of benthivorous fish reduced grazer biomass and thus increased periphyton biomass, indicating a fish-induced three-level trophic cascade, supporting **hypothesis 1a**.

- The strength of periphyton top-down control varied in temporally shaded streams due to seasonal shifts in light supply, confirming **hypothesis 1b**. Top-down control was strongest during autumn (periphyton growth was

light-limited), and weaker during spring (no light-limitation). Primary production varied strongly across the season whereas the grazing rate is more or less constant indicating a temporal asymmetry.

- The expectation that benthic grazers would structurally alter the periphyton physiognomy was not met because grazing mayflies were not able to reduce overstory canopy and therefore **hypothesis 2** has to be rejected.

- Supporting **hypothesis 2a**, the presence of benthivorous induced an alteration in periphyton community composition due to biomass changes of grazer species which prefer certain algal food items. Total diatoms benefited from fish presence, whereas the fraction of stalk/tube forming diatoms was reduced due to biomass increase of those grazer species which preferred this specific diatom group.

- The presence of benthivorous fish did not change feeding selectivity of benthic grazers and therefore **hypothesis 2b** has to be rejected.

- **Hypothesis 2c** was supported because in presence of nocturnal benthic feeding fish (stone loach and gudgeon) benthic grazer species *B. rhodani* altered its temporal feeding pattern from nocturnal to diurnal in order to avoid this predator type.

- A reduction of food intake by *B. rhodani* as consequence of its predator avoidance behaviour could not be clearly demonstrated, not confirming **hypothesis 2d**.

5.2 Ecosystem implications and concluding remarks

Mayfly larvae as dominant grazers in the studied systems were not the optimal grazer species for removing algal overstory (filamentous growth forms) and stimulating an understory periphyton mat. It is difficult to accurately predict the intensity of grazer-periphyton interaction in running waters because it depends on specific characteristics of a periphyton assemblage, such as taxonomy, physiognomy, developmental stage, chemical composition or local habitat and on the traits of the grazers involved, such as body and mouthpart morphology, mobility, feeding

mode, behavioural adaptations and grazer density (Gregory, 1983; Lamberti and Moore, 1984; Hill and Knight, 1987). Fluctuating environmental conditions in stream ecosystems influencing both benthic grazers and periphyton further increase this uncertainty. To ensure removal of crucial algal layers which are largely responsible for the clogging process in the hyporheic zone, specific traits of the ambient grazer community should carefully be assessed in each system.

The reason why studied heptageniids failed to effectively control periphyton overstory might be found in their habitat preferences. The family Heptageniidae includes many indicator organisms for very high or high biological water quality, sensitive to various forms of pollution (Elliott et al., 1988) and abundant in small streams. Periphyton in the small streams studied is dominated by understory growth forms contrary to the periphyton in larger streams and small rivers which have naturally higher nutrient concentrations and allow development of overstory growth forms. Therefore it can be assumed that grazer communities of each system are specialised on the dominating periphyton composition. In other words, heptageniids are well adapted to feed on algal understory while grazer communities in higher order streams are more effective in removing filamentous growth forms, naturally prevailing there.

Although top-down control on periphyton was evident within the studied stream ecosystem, its strength was not sufficient to reduce the periphyton biomass peak in spring. This was mainly caused by the seasonality of temperate systems, where abundance and identity of periphyton change and predatory effects on non-adjacent trophic levels vary to the same extent (Kishi et al., 2005; McIntosh et al., 2005). A stronger top-down control might be expected in unshaded stream ecosystems. Based on the results available, the absence of shading could lead to a reduction of seasonal variations in periphyton growth and thus, resulting in a more stable periphyton biomass which would allow development of permanent high grazer biomasses and more constant use of primary production (Katano et al., 2007; Buria et al., 2010). Consequently, benthic grazers would not be temporally limited by their resources (Dobson and Hildrew, 1992; Wallace et al., 1999) in such streams.

Benthic algae themselves show short-term temporal biomass dynamics regardless of streamside vegetation. Benthic algal biomass accrual generally follows a short-term pattern from commencement of colonisation over the maximum accrual biomass to the phase of biomass loss (Biggs, 1996). Moreover, among the periphyton

community the process of succession occur often, beginning with an organic matrix and bacterial flora over the transition of small prostrate diatoms over attached colonial diatoms to filamentous green algae (e.g. Hudon and Bourget, 1981; Korte and Blinn, 1983; Peterson and Stevenson, 1990). However, these short-term biomass dynamics can be masked by variability when sampling is done at a too small or too coarse scale resulting in problems of quantifying biomass dynamics in a stream system (Biggs, 1996). Spatial variations in hydrodynamic conditions (Biggs and Stokseth, 1996) and grazer densities (Downes et al., 1993) also cause variations in periphyton growth and loss resulting in a patchy biomass distribution. When evaluating strength of top-down control on periphyton, seasonality as well as successional patterns and spatial variability of periphyton biomass should be considered to ensure the success of the measures.

Often nutrient inputs are not the only stressors affecting structure and function of running water ecosystems. Any application of the biomanipulation approach should also consider interactions of nutrients with other natural and anthropogenic stressors (e.g. suspended sediments, pesticides, climate change) and their combined effects. Complex stress regimes on aquatic ecosystems can have far reaching ecological effects such as impacts on ecosystem functioning, mediated by changes in biodiversity and benthic communities (Breitburg et al., 1998; Schindler, 2001; Vinebrooke et al., 2004). Hence, possible impacts of combined stressors on key stone species physiology and behaviour can potentially result in a less efficient food web manipulation due to the impairment of biomanipulation target species. There remains an urgent need to deepen the general knowledge of interactive effects of multiple stressors on ecosystems to predict their responses to a changing environment and to understand the complex impacts cascading through stream food webs.

Especially exposure to pesticides from agriculture can reduce the grazing performance in running waters and thereby affect the effectiveness of biomanipulation (focusing on the reduction of periphyton biomass). Insecticides such as 'lambda-Cyhalothrin' can, for instance, reduce grazing performance via grazer mortality or behavioural changes, which consequently result in increasing periphyton biomass (Rybicki, 2014). Even exposure to low concentrations of the herbicide 'Terbutryn' induced a trophic cascade, which indirectly influenced the grazer species *R. semicolorata* due to clearly reduced growth and energy storages of these grazers (Rybicki et al., 2012). Pesticides are widely used in agriculture and occur in streams in

different combinations. It can be expected that they will negatively affect the ecosystem service 'benthic grazing' in some form, and will consequently impair biomanipulation of stream ecosystems.

Concerning future research priorities in the field of eutrophication control in running waters, several factors have to be taken into account. Mayflies are not the only grazers within an ecosystem. Other grazer species such as caddisflies (e.g. Limnephilidae, Glossosomatidae) and gastropods (e.g. Planorbidae) are known to be more effective in removal algal overstory (Steinman et al., 1987a). The combination of different grazer types might have stronger effects on periphyton biomass as indicated by experiments, where two grazer types (mayflies and snails) decreased the periphyton biomass significantly stronger than each single type (Hertonsson et al., 2008). Interspecific competition can also affected habitat selection of grazers and therefore change spatial patterns of grazing pressure (Hertonsson et al., 2008). Hence, competition can also be assumed to be an important structuring factor in macroinvertebrate communities and consequently, grazer species composition can be crucial for the effectiveness of biomanipulation.

Some general factors should be considered in order to successfully transfer the biomanipulation concept from standing to running waters: stream orders, regional distinctions, differently used catchment areas and the cooperation of all stream users (e.g. authorities, fishery managers, fishermen, residents). Ultimately, requirements which determine the success or failure of the biomanipulation approach need more attention and can be only understood by experimental manipulation of stream ecosystems. Besides application in stream and river protection, the research on biomanipulation substantially contributes to the progress in understanding trophic interactions in complex stream food webs.

6 Summary

A good ecological status of streams and rivers is crucial for maintaining ecological functionality of running waters. Worldwide eutrophication threatens to change structure and function of freshwater ecosystems (Dodds et al., 2008). To reduce the symptoms of eutrophication in streams and rivers an additional approach, besides the reduction of external nutrient inputs from catchment areas, is needed. Therefore the goal has been set to transfer the approach of biomanipulation, which is widely accepted as tool in water-quality management in lakes and reservoirs, to streams. The objective of this study was accordingly to analyse and evaluate some crucial preconditions for top-down control of stream food webs. For that purpose the present thesis examined effects of fish predation (stone loach and gudgeon) on grazer-periphyton interaction in small streams by assessing predator avoidance by benthic grazers, effects of benthic grazers on periphyton community composition during fish presence/absence and the possibility of top-down control on algal biomass by benthivorous fish.

The results demonstrated that similar to lakes and reservoirs top-down acting ecosystem-internal mechanisms are present in small stream ecosystems (see **Hypothesis 1**). The occurrence of a trophic cascade from benthivorous fish via benthic grazers to periphyton with indirect positive effects of fish on periphyton biomass was one of the major results of this study (see **Hypothesis 1a**). A successful control of stream food webs in the sense of biomanipulation is only effective when this principal precondition is fulfilled. However, the strength of top-down control varied seasonally as indicated by scenario analysis using a dynamic model (see **Hypothesis 1b**). A stronger grazing pressure was observed during summer and autumn than during spring. The reason for this was seasonal change of primary production whereas the grazing rate was more or less constant throughout the year. Large differences in generation length (mayflies one year, algae only few days) led to the temporal asymmetry of these processes which finally weakened top-down control on periphyton in temporal shaded streams.

Besides reduction of periphyton biomass, the structural change of periphyton community, removing algal overstory (filamentous and chain/rosette forming algae) and establishing an adherent algal understory (attached/motile and stalk/tube forming algae) is an important objective of a successful biomanipulation as well.

However, dominant grazer taxa (Ephemeroptera: *Baetis rhodani*, *Electrogena ujhelyii*, *Rhithrogena semicolorata*, *Ecdyonurus* sp.) in the streams failed to control periphyton structure as desired by reducing overstory algal species because they avoided filamentous growth forms within the periphyton matrix (see **Hypothesis 2**). Consequently, even high mayfly densities did not promote a thin and adherent periphyton mat. This finding means that the second crucial precondition, the ability to control filamentous algae to reduce clogging (colmation) of the hyporheic zone and facilitate water exchange in eutrophic stream ecosystems (Ibisch et al., 2009), was not achieved by the prevailing grazer community.

Nevertheless, benthivorous fish affected the periphyton community composition via benthic grazers (see **Hypothesis 2a**). This was caused by a combination of density-mediated indirect interaction (reduction of grazer biomass) and trait-mediated interaction (shifts in feeding behaviour of the grazers). Diatoms benefited from fish presence because fish reduced the total grazer biomass. Mayfly species strongly preferred diatoms and thus, grazing pressure on diatoms decreased during fish presence. In contrast the fraction of stalk/tube forming diatoms was heavily reduced in the presence of fish. The grazing pressure on this diatom group increased because the biomass of two mayflies (*B. rhodani*, *Ecdyonurus* sp.), which preferred stalk/tube forming diatoms, increased. These facts illustrate the high importance of fish-induced changes in grazer communities and the difficulty of implementing fish manipulation in streams due to both positive and negative fish effects on specific grazer species. However, the overall food preference of benthic mayfly grazers was not affected by benthivorous fish (see **Hypothesis 2b**).

A shift in feeding behaviour from initially nocturnal to diurnal was observed for the mayfly species *B. rhodani* which resulted in a temporal pattern inverse to that of benthivorous fish (see **Hypothesis 2c**). This shift was interpreted as predator avoidance behaviour due to the high risk of fish predation during night. Associated reductions in grazing rates could not be confirmed (see **Hypothesis 2d**). However, it was evident that *B. rhodani* larvae were highly sensitive to chemical cues related to the actual predation threat of benthivorous fish. Consequently, larvae were able to assess variation in predation risk and alter their feeding habits by flexible behavioural adjustments.

Despite these promising results the applicability of the biomanipulation approach in temporally shaded streams seems to be limited because the asymmetry in

temporal processes weakening top-down control on periphyton. A grazer biomass high enough to reduce the periphyton biomass peak in spring seems to be impossible due to food limitation of benthic grazers during summer time. A short-term biomass increase of benthic grazers during times of high food supply, as for instance observed for *Daphnia* sp. in lakes, is not possible in streams because invertebrate grazer reproduce once or twice per year only.

However, an application of the biomanipulation approach can be recommended for unshaded streams. Based on the results presented here it can be reasonably assumed that absence of shading riparian vegetation would reduce seasonal changes in periphyton biomass. This way, permanently high grazer biomasses resulting in a consistently high grazing pressure can be expected to control periphyton biomass in such stream ecosystems.

In conclusion a correctly adjusted fish stock (suitable species, right density) and careful assessment of specific traits of the ambient grazer community can finally ensure that the ecosystem service 'benthic grazing' achieves its optimal performance and can contribute to control of excessive and nuisance algal growth. It is important to note that the biomanipulation approach serves as additional tool and can be effective only in combination with control of external nutrient loads from catchment areas.

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Appendix

References to own published articles used in the present thesis

***Chapter 2** has been already published with minor alterations under the title:*

Winkelmann, C., Schneider, J., Mewes, D., Schmidt, S.I., Worischka, S., Hellmann, C., Benndorf, J. 2014. Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. Freshwater Biology 59, 803-818. DOI: 10.1111/fwb.12305

The final publication is available at: http://onlinelibrary.wiley.com.

The chapter differs from the final published article in the following respects: Appendix 1 has been inserted as table 1.4 to the method section, text within this section has been newly structured and numbering of tables and figures was adapted to the structure of the thesis.

***Chapter 3:** A manuscript containing the presented results has been submitted for publication under the title:*

Schneider, J., Hellmann, C., Wissel, B., Berendonk, T.U., Benndorf, J., Winkelmann, C. 2015. Grazer-mediated effects of benthivorous fish on stream periphyton community composition.

***Chapter 4** has been already published with minor alterations under the title:*

Schneider, J., Worischka, S., Hellmann, C., Benndorf, J., Winkelmann, C. 2014. Flexibility in feeding periodicity of a grazing mayfly in response to different concentrations of benthivorous fish. Limnologica-Ecology and Management of Inland Waters 45, 24-32. DOI: 10.1016/j.limno.2013.10.002

The final publication is available at: http://sciencedirect.com.

The chapter differs from the final published article in the following respects: text within this section has been newly structured and numbering of tables and figures was adapted to the structure of the thesis.

List of Publications

Schneider, J., Avramov, M., Winkelmann, C., Benndorf, J. 2008. Benthisches Grazing – ein Werkzeug zur Eutrophierungssteuerung in Fließgewässern? Tagungsband der Deutschen Gesellschaft für Limnologie (DGL). *Erweiterte Zusammenfassungen der Jahrestagung 2008* (Konstanz).

Schneider, J., Worischka, S., Hellmann, C., Benndorf, J., Winkelmann, C. 2014. Flexibility in feeding periodicity of a grazing mayfly in response to different concentrations of benthivorous fish. *Limnologica-Ecology and Management of Inland Waters* 45, 24-32.

Winkelmann, C., **Schneider, J.**, Mewes, D., Schmidt, S.I., Worischka, S., Hellmann, C., Benndorf, J. 2014. Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. *Freshwater Biology* 59, 803-818.

Schneider, J., Hellmann, C., Wissel, B., Berendonk, T.U., Benndorf, J., Winkelmann, C. 2015. Grazer-mediated effects of benthivorous fish on stream periphyton community composition. Submitted to *Freshwater Biology*.

Erklärung zur Eröffnung des Promotionsverfahrens

1. Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

2. Bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts habe ich Unterstützungsleistungen von folgenden Personen erhalten:
   Prof. Dr. Jürgen Benndorf, Prof. Dr. Thomas U. Berendonk and Prof. Dr. Dietrich Borchardt.

3. Weitere Personen waren an der geistigen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich nicht die Hilfe eines kommerziellen Promotionsberaters in Anspruch genommen. Dritte haben von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

4. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt und ist – sofern es sich nicht um eine kumulative Dissertation handelt – auch noch nicht veröffentlicht worden.

5. Sofern es sich um eine kumulative Dissertation gemäß § 10 Abs. 2 handelt, versichere ich die Einhaltung der dort genannten Bedingungen.

6. Ich bestätige, dass ich die Promotionsordnung der Fakultät Umweltwissenschaften der Technischen Universität Dresden anerkenne.

……

Ort, Datum

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Jana Schneider

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Individual contributions to the thesis

Kapitel 2

Carola Winkelmann (Erstautor):

- Projektantrag
- Projektkonzeption
- Entwicklung des dynamischen Modells
- Messung von physikalischen und chemischen Gewässerparametern
- Datenanalyse und Statistik
- Verfassen des Manuskriptes

Jana Schneider (Koautor):

- Design und Durchführung von Laborexperimenten (Grazing experiment)
- Beprobung der Gewässer, Sammeln der Versuchsorganismen
- Datenerhebung und Datenanalyse
- Konsumtionsberechnung
- erste Gedanken zur Modellenwicklung
- Ergebnisinterpretation
- Verfassen des entsprechenden Teils im Manuskript
- Korrekturlesung des Manuskripts

Daniela Mewes (Koautor):

- Design und Durchführung von Laborexperimenten (Periphyton gowth experiment)
- Datenanalyse
- Ergebnisinterpretation
- Verfassen des entsprechenden Teils im Manuskript
- Korrekturlesung des Manuskripts

Susanne I. Schmidt (Koautor):

- Unterstützung bei der Entwicklung des dynamischen Modells
- Ergebnisinterpretation
- Korrekturlesung des Manuskripts

Susanne Worischka (Koautor):

- Bereitstellung von Abundanz- und Biomassedaten der Fische
- Ergebnisinterpretation
- Korrekturlesung des Manuskripts

Claudia Hellmann (Koautor):

- Bereitstellung von Abundanz- und Biomassedaten der Grazer
- Ergebnisinterpretation
- Korrekturlesung des Manuskripts

Jürgen Benndorf (Seniorautor):

- Konzeption des Ökosystemexperiments
- Projektantrag

Michelle Weller-Boucher

- Sprachliche Unterstützung

Kapitel 3

Jana Schneider (Erstautor):

- Konzeptionelle Arbeit und Umsetzung des Ökosystemexperiments
- Design und Durchführung von Freilandexperimenten
- Beprobung der Gewässer, Sammeln der Versuchsorganismen und Nahrungsressourcen
- Vor- und Aufbereitung der Proben im Rahmen der stabilen Isotopenanalyse
- Methodenentwicklung zur Bestimmung und Zählung der Algenzellen des Darminhaltes der Grazer
- Taxonomische Bestimmung und Zählung der Diatomeen im Darminhalt der Grazer
- Datenerhebung und Datenanalyse
- Selektivitätsberechnung
- Ergebnisinterpretation
- Verfassen des Manuskriptes

Claudia Hellmann (Koautor):

- Konzeptionelle Arbeit und Umsetzung des Ökosystemexperiments
- Bereitstellung von Abundanz- und Biomassedaten der Grazer
- Ergebnisinterpretation und Unterstützung in der statistischen Auswertung (multivariater Permutationstest)
- Korrekturlesung des Manuskripts

Björn Wissel (Koautor):

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- Ergebnisinterpretation
- Korrekturlesung des Manuskripts
- sprachliche Unterstützung

Thomas U. Berendonk (Koautor):

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- Korrekturlesung des Manuskripts

Jürgen Benndorf (Koautor):

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- Projektantrag
- Bereitstellung von physikalischen und chemischen Gewässerdaten
- Taxonomische Bestimmung und Zählung der Diatomeen
- Statistische Auswertung (Randomised Intervention Analysis, RIA)
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- Korrekturlesung des Manuskripts

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Jeremy Wilkinson:

- Sprachliche Unterstützung

Kapitel 4

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- Konzeptionelle Arbeit und Umsetzung des Ökosystemexperiments
- Design und Durchführung von Freiland- und Laborexperimenten
- Beprobung der Gewässer, Sammeln der Versuchsorganismen
- Weiterentwicklung und Optimierung der Gut-Fluorescence-Methode nach Cowan and Peckarsky (1990)
- Datenerhebung und Datenanalyse
- Konsumtionsberechnung
- Ergebnisinterpretation
- Verfassen des Manuskriptes

Susanne Worischka (Koautor):

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- Bereitstellung von Daten zum Mageninhalt der Fische
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Richard Boucher

- Sprachliche Unterstützung

Photographs (book cover)

Top left:	*Susanne Worischka*
Top right:	*Jana Schneider*
Bottom left:	*Jana Schneider*
Bottom right:	*Susanne Worischka*